Proceedings of the

# XII International Symposium on Biological Control of Weeds

La Grande Motte, France, 22-27 April 2007

Edited by
M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz and B.G. Rector

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# Preface

## Venue and delegates

The XII International Symposium on Biological Control of Weeds was held from 22nd to 27th April 2007 in Southern France. The venue was the Palais des Congrès at La Grande Motte, on the shores of the *Mare nostrum*, the name used by the Romans for the Mediterranean Sea. Two hundred and fifty delegates from 32 countries attended this 5-day symposium.

## Opening ceremony

The symposium was opened on the morning of Monday 27 April 2008, with a welcome to La Grande Motte talk by the Mayor of La Grande Motte, Mr. Henri Dunoyer. This was followed by an introduction to weed and other research activities in the region by Prof. Jacques Maillet, SUPAGRO Montpellier. The opening address, on risk assessment and biological control of weed, was presented by Dr Ernest Delfosse, USDA.

On Sunday evening, before the opening ceremony, a cocktail party was organized for participants and their partners at the Palais des Congrès.

## Sponsors

The organizing committee is very thankful to the sponsors that supported this international event. Their generousity made the event possible and supported the publication of this Proceedings. They were: CAB International (CABI), California Department of Food and Agriculture (CDFA), Commonwealth Scientific and Industrial Research Organisation (CSIRO), Centre de Coopération Internationale en Recherche pour le Développement (CIRAD), United States Department of Agriculture-Agricultural Research Service (USDA/ARS), The United States Army Corps of Engineers, and the European Weed Research Society (EWRS).

## Symposium programme structure

The scientific program was divided into nine themes with a keynote speaker for all except one theme. There were 68 talks and 180 posters.

| Theme chair | Talks and posters | Keynote speakers and titles |
|---|---|---|
| Theme: Ecology and modeling in biological control of weeds | | |
| Andy Sheppard | 9 talks<br>17 posters | Yvonne Buckley: *Is modelling population dynamics useful for anything other than keeping a researcher busy?* |
| Theme: Benefit-Risk – Cost analyses | | |
| Ernest (Del) Delfosse | 8 talks<br>6 posters | Rachel McFadyen: *Return on investment: determining the economic impact of biocontrol programs* |
| Target and agent selection | | |
| René Sforza | 9 talks<br>43 posters | Robert W. Barreto: *Latin American weed-biocontrol science at the crossroads* |
| Pre-release specificity and efficacy testing | | |
| Hariet Hinz | 7 talks<br>33 posters | none |

| Theme chair | Talks and posters | Keynote speakers and titles |
|---|---|---|
| Regulations & Public awareness | | |
| Dick Shaw | 5 talks<br>8 posters | Ralf-Udo Ehlers: *Regulation of biological weed control agents – results of Policy Support Action REBECA.* |
| Evolutionary processes | | |
| Ruth Hufbauer | 7 talks<br>8 posters | George K. Roderick: *Biological control meets evolutionary biology in the South of France.* |
| Opportunities and constraints for biological control of weeds in Europe | | |
| Paul Hatcher | 5 talks<br>5 posters | Maurizio Vurro & Harry Evans: *Opportunities and constraints for biological control of weeds in Europe.* |
| Release activities and post-release evaluations | | |
| Rosemarie De Clerck-Floate | 10 talks<br>40 posters | Simon V. Fowler, et al.: *Release strategies in weed biocontrol: how well are we doing and is there room for improvement?* |
| Management specifics, integration, restoration, implementation | | |
| John Hoffmann | 9 talks<br>20 posters | Joe M. DiTomaso: *Integration of biological control into weed management strategies.* |

Six workshops were also organized during the week:

1: Brassicaceae weeds by Hariet Hinz & Mark Schwartzlander.
2: Risk assessment by Ernest (Del) Delfosse.
3: Aquatic weeds by Michael Grodowitz.
4: Feasibility of biological control of common ragweed (*Ambrosia artemisiifolia*) in Europe by Dominique Coutinot , Massimo Cristofaro , Levente Kiss & Pierre Ehret.
5: Rearing insects by Rosemarrie De Clerck-Floate & Hariet Hinz.
6: Swallow worts by Lindsey Milbrath.

Reports on two of these workshops (Biological control of ragweed, and Rearing insects) can be found at the end of this proceedings.

## Mid-symposium tours

Two options were given to delegates: A visit to the Cévennes (foothills of the Massif Central) or to the Camargues (delta wetlands of the Rhône River). Both tours were held on the sunny day of Wednesday 24 April. The Camargues tour was organized by Marie-Claude Bon and Brian Rector, and 200 delegates visited this natural reserve and enjoyed seeing local fauna, such as black bulls, white horses, Grey Heron, greater flamingos, under the guidance of Nicolas Beck from the Tour du Valat Research Center. Special attention was given to invasive *Baccharis sp.*, Pampa's grass, *Ludwigia* spp. The Cévennes tour was organized by Janine Vitou, Mic Julien and René Sforza. One hundred delegates visited a part of the only French national park in the low mountains. This included a short walk along an ancient Roman road and a scenic picnic. The park guide, Emeric Sulmont, discussed the negative impacts of the invasives *Fallopia japonica* and *Robinia pseudoacacia* and the control methods conducted by local authourities.

## Wine and cheese evening and gala dinner

On the evening of Tuesday 23 April, a wine and cheese party was held. The choice was a selection of succulent and delightful cheeses of France picked by our specialist, Thierry Thomann. The cheese was accompanied by red and white wines, and other interesting beverages, from all over our planet, brought by the delegates. It was a memorable evening with almost no cheese and wine remaining afterwards.

The conference dinner was held on the evening of Thursday 26 April at Le Château du Pouget, located at Vérargues, with historical significance and romantic ambience. After welcome drinks and buffet in the park of the 11th century Château a dinner was accompanied by musical entertainment from the band *Agate ze bouze*. Poster and oral presentation prizes were awarded during the evening.

## Committees and support

The local organizing committee comprised Janine Vitou, Marie-Claude Bon, Brian Rector, Mic Julien (co-chair), René Sforza (co-chair) and Andy Sheppard.

The scientific committee comprised Mic Julien (convenor), René Sforza, Marie-Claude Bon, Brian Rector, Matthew Cock, Massimo Cristofaro, Paul Hatcher, Hariet Hinz, Walker Jones, Thomas Le Bourgeois, Hélia Marchante, Heinz Müller-Schärer, Marion Seier, Richard Shaw, Andy Sheppard, and Janine Vitou. Conference administration was provided by AlphaVisa Congrès. Additional secretariat services was given by Sarah Hague, and computer logistics was supported by Xavier Chataigner. Léo Ruamps, Benjamin Gard, Christophe Girod and Steeve Schawann helped with logistics.

The editorial panel for this proceedings comprised Mic Julien, René Sforza, Marie-Claude Bon, Harry Evans, Paul Hatcher, Hariet Hinz and Brian Rector.

## Next symposium

The attendees agreed that the next meeting should be held in Hawaii, USA. It will be convened by Dr Tracy Johnson, USDA Forest Service.

*René Sforza*
USDA-ARS-EBCL

*Mic Julien*
CSIRO European Laboratory

Local committee (left to right): Brian Rector, René Sforza, Mic Julien, Janine Vitou, Marie-Claude Bon.

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Theme 1:

# Ecology and Modelling in Biological Control of Weeds

## Session Chair: Andy Sheppard

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## Keynote Presenter

# Is modelling population dynamics useful for anything other than keeping a researcher busy?

**Y.M. Buckley[1,2]**

*Summary*

Understanding and modelling the population dynamics of weeds and/or biological control agents can require large investments of time and money; just what are we getting for our modelling efforts? Here I respond to three persistent critiques of modelling as used in biological control programmes and present new directions for extending and improving our use of models. Complex models have been critiqued as resource-intensive, too narrow in scope and difficult to analyse, whereas simple, strategic models are critiqued as oversimplified and inaccurate in predicting postinvasion population dynamics. I argue that models across this spectrum can be useful and that the dichotomy between simple and complex models can be broken down. Biological control practitioners often operate in systems with a high degree of stochasticity and uncertainty; therefore, the incorporation of stochasticity and uncertainty into population models is essential for the development of robust management strategies. Close dialogue between managers and modellers is essential for the application of modelling studies to management. New directions for modelling in biological control include the incorporation of invader impact and complex ecosystem effects such as habitat heterogeneity and disturbance. The right model used for the right question can bring us insights into the biological control process that would be difficult or impossible to achieve otherwise.

***Keywords***: population dynamics, modelling, biological control.

## Introduction

*"Working out the population dynamics of a species can keep a large research group going for a long time. This is generally not possible in a biological control program"* (Zalucki and van Klinken, 2006).

Although Zalucki and van Klinken (2006) refer specifically to the use of population modelling for predicting biological control agent abundance across their potential exotic ranges, I have used this quote to represent a common critique of modelling projects, which is that they are time- and data-hungry, too simplistic and contribute little of use to on-ground managers. The use of different kinds of models to inform and evaluate weed biological control programmes has become well-established in the past decade (e.g. Rees and Paynter, 1997; Shea and Kelly, 1998; Buckley *et al.*, 2005b), but critiques remain on the general use of models, the questions they are brought to answer and the applicability of their results for management. Here I address three critiques of population modelling and identify directions where modelling tools are likely to generate useful new insights into the role of biological control in weed management.

## Critiques of population modelling

Three common critiques of population modelling as a component of biological control programmes are discussed here:

1. model complexity and simplicity (covering both detail and stochasticity);
2. uncertainty in model structure and parameters; and
3. applicability of modelling studies to on-ground management.

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## Model complexity and simplicity

One of the primary axes along which different types of model can be ranged is that which at one of its extremes has tactical, complex, predictive models and at the other has strategic, simple, general models of little predictive power in specific cases. Both extremes have been criticized in relation to their value in biological control programmes, with tactical models critiqued as being resource-intensive (Nehrbass and Winkler, 2007), too narrow in scope and difficult to analyse (Schreiber and Gutierrez, 1998), whereas strategic models are critiqued as oversimplified and inaccurate in predicting postinvasion population dynamics (Zalucki and van Klinken, 2006). Models right across the spectrum have been critiqued as inadequate in contributing to management solutions. It should be noted that this is a long-standing general debate in applied ecology and is not confined to the field of biological control. Models right across this axis of complexity can be badly and well-applied and the ability of the model to contribute to understanding and solving the driving problem should be the criterion used for judging the success of the modelling approach. In other words, the type of model to be used depends on the question being asked. The availability of data to validate and test models is also important, and closer dialogue amongst modellers, biological control practitioners and empirical biological control researchers will lead to more appropriate modelling approaches and collection of data necessary for such models. The aim of most modelling studies is not to reproduce exactly the dynamics seen in the field but to test hypotheses about how we believe the system to be working. Ability to exactly reproduce field dynamics should not necessarily be the 'acid test' of the success of a modelling approach. For example Buckley *et al.* (2003) constructed a complex individual-based model of *Hypericum perforatum* L. (Clusiaceae) dynamics that incorporated biotic and abiotic drivers of dynamics as well as habitat differences and characterized the stochasticity in the system at several spatial and temporal scales. However, despite its 'realism' and ability to accurately represent the structure of field populations, it was not possible to predict dynamics in the field. The aim of this model was to produce virtual populations of plants that behaved like *H. perforatum* plants on which management strategies could be tested. The incorporation of stochasticity was important to determine how robust the management strategies would be to the variability observed in the field.

Stochasticity is variability in population model parameters or structure due to underlying processes such as spatial or temporal variability, e.g. effects of weather on seed production may give rise to a distribution of fecundity values through time. Stochasticity cannot be reduced by applying greater empirical effort, and we may come to know the distribution of values more precisely, but no amount of measurement will reduce the yearly fluctuations in seed production. We know that population dynamics vary in space and time and that the effect of biological control agents is also likely to vary; purely deterministic models will therefore fail to predict the results of the interaction over the range of conditions likely to be encountered in the field. Does this mean that deterministic models should be abandoned? I would argue the contrary, as traditional analysis of deterministic models gives an indication of the likely dynamics under a range or all possible parameter values.

Buckley *et al.* (2005b) used a deterministic, coupled, plant–herbivore model to explore the qualitative population dynamics likely to result from the interaction of the weed *Echium plantagineum* L. (Boraginaceae) and the weevil *Mogulones larvatus* Schultze (Coleoptera: Curculionidae). Ideally, classical biological control would result in a reduced but stable population of the weed supporting a stable population of herbivores; large population fluctuations of either the weed or herbivore could lead to extinction of the weevil and subsequent loss of control. Stability boundary analysis of deterministic models enables identification of the parameter values that give rise to stable, as opposed to oscillatory, dynamics. These ideal parameter values can then be compared with estimates from the field or laboratory. The central critique of studies such as this one is that factors other than intrinsic population dynamics regulate populations and that stochastic effects of spatial or temporal variability could dampen or enhance oscillations resulting from the intrinsic deterministic dynamics alone. This criticism is entirely valid, but in the *Echium–Mogulones* case, despite the deterministic origins of the model, it proved possible to reproduce reasonably well the qualitative and quantitative dynamics in the field observed over seven years (data not shown), and field densities of plants predicted by the model before and after introduction of the biological control agent corresponded well with observed data (Buckley *et al.*, 2005b).

We should expect reasonably tight linkage between agent and weed dynamics where the biological control agent has a strong effect on the plant. As the agents are host-specific, their resource base is greatly simplified, and in the case of *M. larvatus*, it lives within stems, with larvae competing strongly with each other for the plant resource, leading to strong density dependence driving the dynamics. Coupled plant–herbivore models are very rarely explored in a biological control context (Barlow, 1999), so it is currently difficult to predict what kinds of dynamics are likely to result from different biological control agent species (e.g. from various taxonomic groups, feeding guilds). We do not know in which cases strong intrinsic dynamics are likely to drive the interaction or in which cases stochastic factors will overwhelm any deterministic pattern.

## Uncertainty in model structure and parameters

Uncertainty differs from stochasticity in that it represents unknown parameter values, distributions or model structure; it represents the extent of our ignorance of a system. Uncertainty may be reduced through the collection of more data, but commonly in invasive plant studies, we cannot afford to invest the time or resources necessary for intensive data collection before management decisions are made. Even when detailed data are available, it may still be impossible to determine the correct model to use (e.g. for *E. plantagineum*, both scramble and contest competition models fit the data equally well for *M. larvatus* density dependence; Buckley *et al.*, 2005b). Methods for including both parameter and model uncertainty into population models are therefore highly relevant but relatively underused in invasive plant management models.

Parameter uncertainty is pervasive and often unacknowledged; only rarely can we determine parameter estimates with sufficient confidence whilst representing all sources of stochasticity accurately. Buckley *et al.* (2005a) provided an example of a population and spread model of an invasive pine species, *Pinus nigra* Arnold, with a high degree of uncertainty in the demographic and dispersal parameters. Traditional matrix (for population growth) or integro-difference equation (for spread) models are run under one or a few parameter scenarios. Subsequently calculated sensitivities and elasticities then inform management by highlighting parameters and life history stages to target for control. However, the particular parameter values used will change the pattern of sensitivities and elasticities for population growth rate or spread (Caswell, 2001). Buckley *et al.* (2005a) investigated whether, given a range of possible values, there are consistent patterns that can be exploited for robust management. Despite the large range of uncertainty identified in this case, consistent patterns of sensitivities and elasticities with non-overlapping confidence intervals did emerge. This enabled the identification of suitable robust management targets in a number of different habitats. Buckley *et al.* (2005a) used a Monte-Carlo sampling approach to incorporate uncertainty; other suitable methods that should be explored are information gap theory (Ben-Haim, 2001) and uncertain number theory (Regan *et al.*, 2004).

## Applicability of modelling studies to on-ground management

To date, we have had some successes in the use of models to inform management strategies in the field. Buckley *et al.* (2004) used a model of *Mimosa pigra* L. population dynamics to make recommendations about the type of integrated weed management (IWM) strategy that would have the greatest effect on the reduction of *M. pigra* population size over 3 years. The role of biological control in this IWM strategy was found to contribute substantially to its success. IWM strategies are relatively complex, and their results may be unpredictable because of population processes and interactions between individual control techniques. In such cases, the use of models is quite germane but still surprisingly rare.

Buckley *et al.*'s (2005a) study of the population dynamics of *P. nigra* was initiated by a management-driven question about whether the introduction of a seed-feeding biological control agent would have the potential to reduce the rate of spread of the invasive pine. As spread speed was found to be relatively insensitive and inelastic to the fecundity parameters, initial recommendations were that a seed feeder would not be highly appropriate. Modelling studies are increasingly important in the prerelease phase of biological control programmes where the weed dynamics and vital rates are examined for potential management targets (Davis *et al.*, 2006).

# New directions

We can do more to increase the applicability of our models to management. Incorporation of impact and ecosystem effects into population models may have important implications for biological control programmes.

## Impact

Impact is what separates troublesome invaders from the merely naturalized, and the importance of including nonlinear, density–impact relationships in biological control studies has recently been recognized (Thomas and Reid, 2007). To date, impact has rarely been broached in management models of invasive plants. It has implicitly been assumed that a reduction in density will lead to a corresponding reduction in impact. If however, impact is nonlinearly related to population density (see Fig. 1 in Thomas and Reid, 2007) and varies amongst weed species, a biological control agent causing only a small reduction in one weed species' density may be more effective at reducing impact than another biological control agent having a large effect on a second weed species' density. If we assume a linear weed density–impact curve that it is in fact nonlinear, we may be incurring large costs, in both lack of impact and overinvestment in ineffective or wasted control efforts.

## Ecosystem effects

Nonparametric time-series analysis of the dynamics of the interaction between cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), and its host plant, ragwort, *Senecio jacobaea* L. (Asteraceae), revealed strikingly different dynamics in two different locations

(Bonsall *et al.*, 2003), demonstrating that environmental context can determine the strength of intrinsic dynamics. Several studies show the habitat specificity of population dynamics, management actions and/or biological control agents (Buckley *et al.*, 2003, 2005a; Shea *et al.*, 2005; Davis *et al.*, 2006), as plant population dynamics differ between locations even within an invaded range. It is also apparent that plant population dynamics and hence management will be affected by disturbance regimes, whether natural or anthropogenic, including those caused by weed management itself (Buckley *et al.*, 2004, 2007). The inclusion of broader ecosystem effects in population models is therefore highly relevant for management.

## Conclusions

Although critiques of the use of population modelling in biological control programmes remain, I believe that we have had some success in improving management strategies before release of agents and in determining the potential for success in ongoing biological control programmes. My research group also plans to use models to retrospectively evaluate the effect of biological control in a historical biological control programme. Future progress in the use of modelling in biological control programmes will be in the use of established techniques earlier in the programme (e.g. prerelease), the incorporation into population models of measures of impact of the agents on the weed and of the weed on the affected ecosystem or industry and the incorporation of broader ecosystem effects on the population dynamics of the weed and the biological control agent. Models from across the spectrum of complexity to simplicity can be useful at different stages in a biological control programme. The incorporation of uncertainty directly into the models will enable us to focus on robust management strategies that are not contingent on a narrow set of parameters or model structure assumptions.

## Acknowledgements

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# Biomass reduction of *Euphorbia esula/virgata* by insect/bacterial combinations

**A.J. Caesar[1] and R.J. Kremer[2]**

*Summary*

Biological control efforts against the perennial invasive *Euphorbia esula/virgata* in North America have left 30–50% of all treated sites without impact after 10–15 years. Those efforts focused almost exclusively on insect releases. Much evidence is available indicating that soil biotic factors affect both invasiveness and biocontrol effectiveness. The authors have shown that soilborne bacteria and fungi are linked to biomass reductions or mortality in conjunction with insect damage. To understand factors possibly affecting synergistic interaction of the insects with plant pathogens shown to cause rapid weed mortality, predominant bacteria associated with the flea beetle *Aphthona flava* Guill. (Coleoptera: Chrysomelidae) released to control *E. esula/virgata* L. in western North America, were isolated and identified. Two *Euphorbia*-infested sites with widely differing levels of impact 8–10 years after insect release were sampled. From the site that exhibited rapid, sweeping declines in *Euphorbia* density, 6 of 12 isolates were *Bacillus* spp., 4 were coryneform species and 2 were *Pseudomonadaceae*. Bacteria isolated from the Cottonwood site included some species often associated with the biocontrol of soilborne plant pathogens. The results of tests for a range of hydrolytic enzymes showed that the two groups differed in the frequency of isolates positive for such enzymes as cellulase and xylanase. Two isolates from each location representative of predominant bacterial species and their range of traits were selected for testing on *E. esula/virgata* in combination with *Aphthona* spp. After 35–37 weeks, two isolates positive for cellulase from the Knutson Creek site caused significant ($P$ = 0.05) dry weight reductions of *E. esula/virgata* plants of 64% and 67%, respectively, in combination with *Aphthona* spp. One of the two isolates from the Cottonwood site, also positive for cellulase production, caused a 60% reduction in dry weight compared with the control.

***Keywords***: trophic interactions, synergism, biological control, bioherbicides, bacteria.

## Introduction

The hypothesis addressed in this work is whether the degree of biological control activity of the flea beetle *Aphthona flava* Guill. (Coleoptera: Chrysomelidae) on the perennial invasive prairie plant, *Euphorbia esula/virgata* L. (leafy spurge) is associated with traits within members of the bacterial community vectored by the beetle. It is not known whether the microflora associated with the flea beetles contains species that could affect *E. esula* by either acting as antagonists against the documented plant pathogens or enhancing pathogenicity through accentuating tissue degradation. Previous studies by the senior author have shown that the effective biological control at the Knutson site was because of the presence and action of *Rhizoctonia solani* Kuhn and *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen that were isolated from plants at that site. These fungal species, obtained from insect-damaged tissue of *E. esula/virgata*, were shown to be highly virulent either independently (Caesar, 1994, 1996) or in combination with *Aphthona* spp. (Caesar, 2003). Hydrolytic enzymes were chosen as the traits of interest because of their potential for increasing plant tissue damage as well as conversely acting against soilborne pathogens through lysis of fungal hyphae. Bacterial isolates were tested for hydrolytic enzyme production to determine whether there were trends in enzyme spectra amongst isolates from beetles recovered at a successful biocontrol site and isolates from a less successful release site.

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Previous studies by Kremer have documented deleterious rhizobacteria that can damage *E. esula* (Kremer and Kennedy, 1996; Kremer *et al.*, 2006).

# Materials and methods

## Plant propagation

Plants used in this study were propagated from cuttings of plants obtained from a single *E. esula/virgata* infestation in northeast Montana. Plants weighing ca 30 g or more were selected for the experiment, after being produced through continuous culture over more than 1 year and were of an overall size nearest to typical field-size plants as was achievable in the greenhouse whilst retaining a degree of apparent vigour similar to that observed in the field. Plants were grown in the greenhouse at 20–28°C in a potting medium containing equal volumes of peat and vermiculite in 15 × 15 cm (diameter × height) plastic pots.

## Source and collection of *Aphthona* spp. and associated bacteria

To ascertain whether adults of *Aphthona* spp. might vector plant pathogenic bacteria, active adults of the flea beetles *Aphthona nigriscutis* Foudras and *Aphthona lacertosa* (Rosenhauer), were collected using sweep nets from two sites within the Theodore Roosevelt National Park, located in western North Dakota. One site, a portion of the flood plain of Knutson Creek, experienced dramatic reductions in stand density of *E. esula/virgata* following establishment of the flea beetle *A. lacertosa* and attainment of high populations of the insect. Another site, Cottonwood, contained stands of *E. esula/virgata* that had remained apparently unimpacted over several years following releases of *Aphthona* spp. despite establishment of the flea beetle. Half of the *Aphthona* adults collected from each site were washed by placing five adult flea beetles per tube in test tubes (five tubes per lot) containing 9 ml of pH 7 potassium phosphate buffer and vortexing for three 1-minute periods interspersed with pauses of 30 s. Tenfold serial dilutions were prepared from the insect washes and plated on triplicate plates of 0.3% tryptic soy agar (TSBA) and Kings medium B and incubated at 25–28°C. Five apparently distinct colonies were selected from dilution plates on which 20–200 colonies occurred. To include bacteria that might be internal, the beetles of the respective companion lots were washed by vortexing in three changes of a pH 7 phosphate buffer/20% ethanol solution. After the final wash, beetles in groups of five were re-suspended in 9 ml of sterile pH 7 phosphate buffer and ground with a mortar and pestle. Tenfold serial dilutions were plated on media. All cultures were stored over the short term in pH 7 potassium phosphate buffer at 4°C and in Luria–Bertani medium with 15% w/v glycerol at –80°C for long-term storage.

## In vitro tests of bacterial traits

To investigate the effect of phenotypes that included a range or varying intensities of hydrolytic enzyme production might have on the capacity to interact with insect herbivory, hydrolytic enzyme activities of the bacterial isolates were tested using published methods. Filter-sterilized solutions of 0.1 % 4-methylumbelliferyl *N*-acetyl β-D-glucosamine, 0.1% 4-methylumbelliferyl *N*-acetyl β-D-glucosaminide (chitin is a homopolymer of *N*-acetyl-glucosamine; the latter substrate assays for β-*N*-acetylhexosaminidase, a chitin oligosaccharidase), 0.25% *p*-nitrophenyl β-D-mannopyranoside and 0.25% *p*-nitrophenyl β-D-glucopyranoside (Sigma Chemicals, St Louis, MO) (Fahey and Hayward, 1983) in pH 7 phosphate buffer in sterile 96-well microtitre dishes were used to give 150–200 µl per well. Plates were inoculated with isolates and incubated at 20°C for 10–14 days (Santos *et al.*, 1979). Clearing of coloured substrates on agar media during incubation at 20°C for 10–14 days was used in tests to indicate xylanase (Biely *et al.*, 1985) or β-1,4-glucanase (Scott and Schekman, 1980) using 0.2% Remazol Brilliant Blue xylan (4-*O*-methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R) (Biely *et al.*, 1985) and 0.2% Ostazin Brilliant Red hydroxyethylcellulose (hydroxyethylcellulose dyed with Ostazin Brilliant Red H-3B) (both from Sigma Chemicals), respectively, in 2YT medium (Sipat *et al.*, 1987) with 1.5% agar. Tests for polygalacturonase (Hankin and Lacy, 1984) and cellulase (Barros and Thomson, 1987) were also performed. Isolates were also assessed for *in vitro* antibiosis against two soilborne fungal pathogens of *E. esula*: a *Pythium* spp. isolate and a *R. solani* isolate. Bacteria were streaked near the edge of Petri dishes containing 0.3% TSBA, and immediately thereafter, agar plugs taken from colony margins of one of the fungi were placed at the opposite side of plates. Plates with these bacterial/fungal pairings were incubated at 20°C and examined for zones of inhibition after 36 h. Degree of inhibition was scored as –, +, ++ or +++ based on 0, <1-cm, >1- to 2-cm and >3-cm-wide zones of inhibition, respectively.

## Identification by fatty acid methyl ester profiles

Bacterial isolates were identified based on whole-cell cellular fatty acids, derivatized to methyl esters, i.e. fatty acid methyl esters. Isolates from frozen cultures were streaked twice successively on 3% TSBA. After 24 h, cells were harvested and immediately frozen at –20ºC. Fatty acid methyl esters were obtained by saponification, methylation and extraction following the manufacturer's procedure. Bacterial isolates were analysed using the MIDI Microbial Identification Software (Sherlock TSBA40 Library version 4.5; Microbial ID, Newark, DE). The fatty acid methyl ester profile of *Stenotrophomonas maltophilia* (Hugh) Palleroni

and Bradbury (ATCC 13637) was used as a reference for the MIDI determinations. Strains with a similarity index (SIM) ≥0.300 are considered a good match and conclusively identified (Siciliano and Germida, 1999; Oka *et al.*, 2000).

### Tests of insect/microbial interactions on *E. esula/virgata* in the greenhouse

Three isolates from each of the two sites were selected based on traits that broadly typified the respective groups in terms of their taxonomic classification and hydrolytic enzyme spectra. Isolates were grown in TSBA at 20 to 25ºC. Plants of appropriate size and mass were grown as described above. Cages consisting of nylon netting material (32 mesh or 530 lm mesh openings) supported by an aluminum frame were placed over all pots and secured with a clamp to prevent escape of flea beetle adults. Suspensions of isolates selected as described above were adjusted to ca $10^6$ cells per ml and were poured into the potting medium, 200 ml per pot, in which *E. esula/virgata* was growing. Within 24 h of addition of bacteria to the pots, adults of *A. flava* were released, 15 per cage, into the cages. Ten caged plants of *E. esula/virgata* were treated with each bacterial isolate used, and the experiment was repeated once. Treated plants were grown in the greenhouse at 25–30ºC for 35–37 weeks, dried at 47ºC for 10 days upon harvest and weighed. Data were tested to confirm homogeneity of variances (Bartlett and Kendall, 1946) before pooling data from both trials for analysis using Waller and Duncan's exact Bayesian *k*-ratio least significant difference rule ($P = 0.05$) (Waller and Duncan, 1969).

## Results and discussion

Two of the nine assayed of isolates, whether originating from the highly impacted Knutson Creek site or the static Cottonwood site, had a similar average number of positive tests of hydrolytic enzymes (Table 1). However, 6 of 12 Knutson Creek isolates were positive for a suite of three hydrolytic enzymes, β-*N*-acetylhexosaminidase, a chitin oligosaccharidase and two apparently distinct or dissimilar cellulases (all three degrade β-1, 4 sugar residues), whereas only a single isolate amongst the 12 from adults collected from Cottonwood were positive for these three enzymes. Only the three isolates with this suite of three enzymes, including two from Knutson Creek amongst the six isolates tested from the two sites caused significant reductions, ranging from 61% to 67% (Table 2) in dry weight of *E. esula/virgata* in greenhouse tests. The two isolates tested that had little or no hydrolytic enzyme production (identified as *Ochrobacterium anthropii* Holmes *et al.* and *Corynebacterium acquaticum* Lehmann and Neumann) correspondingly failed to reduce biomass of *E. esula/virgata*. Interestingly, an isolate shown to produce only polygalacturonase amongst nine hydrolytic enzymes assayed, identified as *S. maltophilia*, caused a 24% reduction in biomass of *E. esula/virgata*, although this was not significant. *In vitro* antibiosis against *R. solani* and *Pythium* spp. was not a helpful trait in distinguishing the two sets of isolates. The relevance of investigating bacteria associated with adult flea beetles is based on two premises: (1) that the bacteria carried by the flea beetles may be active participants in the phyllosphere and/or rhizosphere once they are carried passively to the plant and (2) that the bacteria found on or in the insects may represent species that predominate in the host plant/insect system. A further possibility is that these bacteria are endemic to the insect or to the plant leaf surface, root zone or perhaps vascular system. Bacteria that have been identified in the few studies done in these realms include species that were identified in the present study: *Ochrobacterum* spp. (Spiteller *et al.*, 2000), *Cellulomonas* Bergey *et al.* 1923, *Microbacterium* Orla-Jensen 1919 (Zinniel *et al.*, 2002), *Bacillus* spp. (Cho *et al.*, 2003), *P. chlororaphis, S. maltophila, B. cepacia* and *Bacillus thuringiensis* Berliner (Canganella *et al.*, 1994). The possibility that bacteria affect herbivory positively or negatively is in need of further exploration and could lead to some important contributions to a better elucidated understanding of biocontrol ecology. That the ecology of classical weed biocontrol is justifiably receiving greater attention seems evident by many contributions to the proceedings of recent International Weed Biocontrol Symposia (Spencer, 2001; Cullen *et al.*, 2004).

Although our results show the effects of the bacteria in reducing biomass of leafy spurge in conjunction with insect damage, a fuller understanding of the potential of such bacteria to cause stand reductions in combination with insects would require application of bacteria in the field following establishment of the flea beetles. Bacteria with the traits we have described are likely accessory to the larger, more pronounced effects of aggressive fungal root and crown pathogens, and they may provide additive effects. We propose to confirm this with further studies by distinguishing the comparative effects of fungi and bacteria. Fungi are two and a half times more likely than insects to be the cause of mortality when assessed using comparative risk survival analysis (Caesar, 2003). It was beyond the scope of this study to show a definitive link of hydrolytic enzyme production and growth reduction. This study did provide indication for simultaneous further screening of additional candidate isolates, using criteria identified here and the immediate testing in the field of selected bacteria, such as isolates producing cellulase or a broad spectrum of hydrolytic enzymes in combination with *Aphthona* spp., for biological control of *E. esula/virgata*. There remain many sites in the field where insects are established without apparent stand reductions where bacteria can be tested. Further, our work has shown that bacterial species not previously considered

**Table 1.** *In vitro* antibiosis and hydrolytic enzyme production by bacteria associated with the flea beetle *Aphthona flava* released at two sites, Knudson Creek site and Cottonwood. Tests for enzymes were with chromogenic substrates.

| Isolate | Phenotypic traits of isolated bacteria[a] | | | | |
|---|---|---|---|---|---|
| | *In vitro* antibiosis vs *Pythium* spp. | *In vitro* antibiosis vs *Rhizoctonia solani* | 0.25% *p*-Nitrophenyl β-D-glucopyranoside test | 0.25% *p*-Nitrophenyl β-D-mannopyranoside | 0.1 % 4-Methylumbelliferyl *N*-acetyl β-D-glucosamine |
| **Knudson Creek site** | | | | | |
| *Pseudomonas putida* 102 | ++ | + | + | + | + |
| *Bacillus cereus* 103 | ++ | – | + | – | – |
| *B. cereus* 104 | – | – | – | – | – |
| *Arthrobacter oxydans* 113 | – | – | – | – | – |
| *Bacillus thuringiensis* 124 | ++ | + | – | – | + |
| *B. cereus* 129 | ++ | + | – | – | + |
| *B. cereus* 154 | ++ | + | – | – | + |
| *Burkholderia cepacia* 207 | ++ | ++ | – | – | – |
| *Corynebacterium acquaticum* 207b | – | – | – | – | – |
| *Cellumonas turbata* 213a | – | – | – | – | – |
| *B. cereus* 216 | + | + | – | – | + |
| *Microbacterium liquefaciens* 223 | +++ | ++ | – | – | + |
| **Cottonwood Creek site** | | | | | |
| *Brevibacterium iodinium* 116 | – | – | + | + | + |
| *Paenibacillus glucoanalyticus* 117 | – | – | – | – | – |
| *Pseudomonas chlororaphis* 217 | ++ | – | + | – | – |
| *Ochrobactrum anthropi* 145 | – | – | + | – | – |
| *Bacillus thuringiensis kurstakii* 146 | + | + | – | – | – |
| *Bacillus cereus* | ++ | + | – | – | + |
| *Pseudomonas putida* 226 | – | – | – | – | – |
| *Pseudomonas chlororaphis* 145 | ++ | – | – | – | – |
| *Stenotrophomonas maltophilia* 144 | – | – | – | – | – |
| No match | ++ | – | + | – | – |
| No match | – | + | – | – | + |
| No match | – | + | – | – | – |

[a] For *in vitro* antibiosis tests, degree of inhibition was scored as: – = no inhibition; + = ≤1-cm-wide zone of inhibition; ++ = >1- to 2-cm-wide zone of inhibition; +++ = ≥3-cm-wide zones of inhibition; NT = not tested. For all other tests: – = trait absent; + = trait present.

| 0.1% 4-Methyl umbelliferyl β-D-glucoside | 0.1% 4-Methylumbelliferyl *N*-acetyl β-D-glucosaminide | Ostazin Brilliant Red hydroxyethylcellulose | Remazol Brilliant Blue Xylan | Polygalacturonase | Cellulase |
|---|---|---|---|---|---|
| + | + | + | + | – | + |
| – | – | – | – | – | + |
| – | – | – | – | – | + |
| – | – | – | – | – | + |
| – | – | + | – | – | + |
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| – | – | – | – | – | + |
| – | – | – | + | – | – |
| – | – | + | – | – | + |
| – | – | – | – | – | – |
| – | – | + | – | – | – |

**Table 2.** Effect on *Euphorbia esula/virgata* of bacteria with various spectra of hydrolytic enzyme production *in vitro* in combination with *Aphthona* spp. Means with different letters are significantly different ($P = 0.05$) as determined using Waller and Duncan's (1979) exact Bayesian *k*-ratio least significant difference rule.

| Origin | Treatment | Mean dry weight (g) |
|---|---|---|
| Knutson Creek | *Aphthona* + *Bacillus thuringiensis* 124 | 16.2 a |
| Knutson Creek | *Aphthona* + *Microbacterium liquefaciens* 223 | 17.4 a |
| Cottonwood | *Aphthona* + *Brevibacterium iodinum* 116 | 19.0 ab |
| Cottonwood | *Aphthona* + *Stenotrophomonas maltophilia* 144d | 37.1 bc |
| Cottonwood | *Aphthona* + *Ochrobacterium anthropii* 145 | 45.2 c |
| Knutson Creek | *Aphthona* + *Corynebacterium acquaticum* 207b | 47.3 c |
| | Control + *Aphthona* | 49.4 c |

amongst those that are deleterious to plant growth can, in combination with insects, cause dramatically negative effects on invasive weed growth compared with insects alone.

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# Rhizosphere bacterial communities associated with insect root herbivory of an invasive plant, *Euphorbia esula/virgata*

**A.J. Caesar[1] and T. Caesar-Ton That[2]**

*Summary*

The invasive perennial plant of Eurasian origin, *Euphorbia esula/virgata*, has been successfully controlled over large areas in North America with a synergism between larvae of *Aphthona* spp. and soilborne plant pathogens. However, a multitude of sites is not yet under control. Studies are needed on how flea beetle root herbivory may alter the microbial ecology of the rhizosphere of *E. esula/virgata* and how the resulting rhizosphere community may affect the synergism. Studies were undertaken at Theodore Roosevelt National Park from 2001 to 2003 to identify the predominant culturable prokaryotic species found in the rhizospheres of *E. esula/virgata*. The hypothesis was that distinct rhizosphere communities of *E. esula/virgata* would be associated with root herbivory by the flea beetle *Aphthona* compared with rhizospheres of *E. esula/virgata* from stands without insect presence. Stands with and without resident populations of *Aphthona* spp. were assayed by spiral plating root washes of *E. esula/virgata* and selecting colonies from the most dilute portion of the spiral (deemed as predominant). Gas chromatographic analysis of fatty acid methyl ester was performed on the resulting pure cultures to identify the isolates and further characterize community structures using principal component analysis. *Pseudomonas syringae* van Hall, *Pseudomonas cichorii* (Swingle) Stapp, *Erwinia chrysanthemii* Burkholder, all plant pathogens, were associated exclusively with herbivory by *Aphthona* flea beetles. Conversely, *Variovorax* Willems *et al.* 1991 and *Aquaspirillum* Hylemon *et al.* 1973 spp. were a greater proportion of predominant species from roots without *Aphthona* present. There were also differences in the occurrence of the root pathogen antagonistic *Pantoea agglomerans* Gavini *et al.* 1989 and *Stenotrophomonas maltophilia* (Hugh 1981) Palleroni and Bradbury 1993.

***Keywords***: synergism, trophic interactions, plant pathogens, soilborne, microbial ecology.

## Introduction

Biological control of plant species of Eurasian origin that are invasive in North American has resulted in considerable success in reducing population densities of several species. One such success concerns the deep-rooted perennial *Euphorbia esula/virgata*, regarded as a fully achieved case of biological control of an invasive plant species. However, the proportion of impacted sites amongst all infested locations has remained at ca 33% (Caesar, 2003; Kalischuk *et al.*, 2004; Hodur *et al.*, 2006). Successful biological control of leafy spurge, characterized by rapid reductions in stand density, is caused by insect/plant pathogen synergisms (Caesar, 2003). Given that the mechanisms driving the successful biological control include soilborne microbes such as plant pathogenic *Fusarium* spp. Link ex Gray, *Rhizoctonia solani* Kuhn and other fungi, possible explanations for the prevalence of unimpacted sites, despite establishment of insect root herbivores, may also be microbial in nature. This aspect has not been investigated previously. It has been increasingly accepted within the field of biological control that microbial interactions are a considerable, significant factor in the biological control of invasive plants (Bacher *et al.*, 2002; Lym and Carlson, 2002; Sing *et al.*, 2005; Butler *et al.*, 2006). This complements a large body of literature showing that exotic plant invasion is both affected by and affects the soil microbial ecology (Belnap and Phillips, 2001; Ehrenfeld *et al.*, 2001; Ehrenfeld, 2003; Kourtev *et al.*, 2002, 2003), including effects on plant succession (Van der Putten

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*et al.*, 1993; Bever *et al.*, 1997). There are also indications that the process of biological control with root herbivore and soilborne microbes, which can be viewed as an accelerated form of negative feedback (Caesar, 2005), may affect patterns of succession following *E. esula/virgata* (Butler *et al.*, 2006). These documented interactions between plants and soil microbes and amongst plants, root herbivores and microbial synergists thus have great implications for biological control, plant succession and restoration of native plant communities. Several questions arise from this body of findings. Concerning the effects of biological control, these include the following:

1. Is there any as sociation between insect (*Aphthona* spp.) damage to roots of *E. esula/virgata* and predominant culturable prokaryote species? Is the lack of stand reduction despite the establishment of insect root herbivores as biological control agents attributable to microbial factors other than plant pathogens?
2. What alternatives are available when large numbers of infestations remain unaffected by the most successful agents and can the percentage of impacted sites be increased through microbial means?
3. How is microbial negative feedback (the accumulation of deleterious microbes in response to individual plant species), shown in a number of cases with invasive plants, manifested in the predominant microbial species that occur in response to insect damage?
   a. How does insect damage to roots of the invasive perennial *E. esula/virgata* affect the structure of prokaryotic microbial communities compared with roots of plants from populations with little or no insect presence?
   b. Do the predominant or prevailing culturable bacteria and actinomycetes from the rhizospheres of populations of *E. esula/virgata* with insect activity act as antagonists to plant pathogens or as low-level plant pathogens?

No previous study has sought to examine effects of root herbivory on the prokaryotic rhizosphere community in relation to biological control of the plant host. Thus, the objectives of this study were to assess communities of culturable prokaryotes associated with rhizospheres of *E. esula/virgata* at sites with heavy flea beetle activity and compare with such communities occurring at locations with no detectable insect activity. We sought to examine and discuss the implications of any trends that the presence of specific bacteria might be indicative of. For example, the presence of certain *Erwinia* spp. would be indicative of soft rot. We hypothesized that prokaryotic rhizosphere communities from populations of the invasive plant *E. esula/virgata* that were damaged by larvae of the flea beetles *Aphthona nigriscutis* Foudras and/or *A. lacertosa* (Rosenhauer) would exhibit considerable distinctions from rhizosphere microbial communities associated with populations without *Aphthona* flea beetle activity. It was surmised that identification of prokaryotes present in the highest numbers would be of critical interest to elucidate their possible role and mode of action in relation to biological control.

Previous studies by the senior author have shown the effects of fungi that were found in insect-damaged root tissue of such invasive species as *E. esula/virgata*, *Acroptilon repens* (L.) DC and *Centaurea maculosa* Lam. But few previous studies have attempted to identify prominent or predominant members of the prokaryotic microflora in response to exotic plant invasion and establishment.

## Materials and methods

Sites within the Theodore Roosevelt National Park in North Dakota, United States, with infestations of *E. esula/virgata* under observation since 1992 were selected for sampling based on the presence or absence of adult flea beetles on the stand, the former status being an indicator of larval attack on the roots earlier in the season, as confirmed by examining roots in work preliminary and subsequent to the work described herein. Five plants within each sampled stand, which ranged in size from, were selected haphazardly for rhizosphere soil samples but were usually 0.5–1 m from the edge of a given stand. Stands ranged in size from 0.2 to 1.2 ha. Three soil cores of 20 cm in diameter to a depth of ca 15 cm containing roots of leafy spurge were taken from around each of the plants. In the laboratory, spurge roots were identified, removed from soil cores and transferred to plastic bags (90 × 160 mm; Intersciences Laboratories, Weymouth, MA) containing 9 ml of pH 7 phosphate buffer and subjected to 1 minute of agitation with a Stomacher 80 (Seward Medical, London, UK). Soil suspensions were plated on 0.3% tryptic soy broth agar (TSBA) medium in triplicate using a spiral plater (Don Whitley Scientific, West Yorkshire, UK). A spiral plating method to serially dilute rhizosphere soil was used to afford a non-random means of selecting colonies from the most dilute portion of the spiral. Plates were incubated at 20–28°C for 3–5 days. Five bacterial colonies found at the end of each spiral were collected from each plate and thus represented the predominant *E. esula/virgata* rhizosphere bacteria for each sampled site (Caesar-TonThat *et al.*, 2007). For identification of isolates, fatty acid methyl ester (FAME) profiles were obtained. FAME profiles are routinely used to identify genera, species and strains of bacteria (Cavigelli *et al.*, 1995; Ibekwe and Kennedy, 1999). In our case, FAMEs were used both as the basis of identification of isolates and further afforded the analysis of intraspecific differences amongst isolates or amongst unidentified isolates with similar taxonomic affinities. FAMEs were obtained by saponification, methylation and extraction following the MIDI system (Microbial Identification System; Microbial ID, Newark, NJ). MIDI Microbial Identifi-

cation Software (Sherlock TSBA50 Library; Microbial ID) was used to identify the isolates. *Stenotrophomonas maltophilia* (ATCC 13637) was used as a reference. Only strains with a similarity index (SIM) of 0.300 were considered a good match (Siciliano and Germida, 1999; Oka *et al.*, 2000). Non-matched isolates were considered conclusively analysed if the percentage of their named peaks was >85%, although they were not assigned identification because of lack of information in MIDI Aerobic Bacteria Library TSBA50. Therefore, they were included in all analyses. The FAME structural classes were categorized into saturated straight-chain fatty acids, branched straight-chain fatty acids, monounsaturated fatty acids and hydroxyl fatty acids. These classes were used as indicators for particular groups of microorganisms (Zelles *et al.*, 1992; Larkin, 2003). The proportion of fatty acid structural classes (expressed in percentage of total fatty acids) were combined from bacterial isolates belonging to a same species or to the same genus (in the case of *Pseudomonas* spp.), and mean values were compared amongst the groups of species. Principal component analysis (PCA) was performed on community FAME data from different treatments (locations with or without insects). The FAME profiles of bacterial isolates were compared by PCA using JMP v6 (SAS, Cary, NC)

The objectives of the present study were to elucidate the bacterial community structure associated with insect herbivory of an exotic, invasive species. Previous studies have focused on key soilborne fungi that are associated with herbivory that have been attributed with causing biological control of invasive plants (Caesar, 2003).

Although there have been studies on above-ground herbivory and soil biodiversity, on the effects of invasive species on soil microbial community structure and on the effects of above-ground herbivory on plant invasion (Maron and Vila, 2001), few studies have examined the effects of root herbivory and rhizosphere microbial community structure. The epicenter of invasiveness may be the rhizosphere interactions amongst plants, microbes and root herbivores. Understanding the effects of specific soil biota can be useful to make predictions about the relative importance of soil organisms in the invasion process, the rate at which stand reductions occur and the likelihood of successful restoration of native plant communities following successful biological control.

The approach taken here of focusing on culturable bacteria is justified on several grounds. Many, if not most of the important parameters that relate to soil and plant health, such as nitrogen cycling, mineralization and soil structure (aggregation, porosity for water holding capacity and respiration), can at present be linked exclusively to culturable soil bacteria. Of the soil microbes known to contribute to such important soil processes as the control of plant diseases, insects and weed pests; beneficial symbiotic associations between bacteria and plants; the recycling of plant nutrients; and the maintenance of soil structure, all are culturable microbes (Caesar-TonThat *et al.*, 2007). Although other species clearly may also play prominent roles in soil biology, the development of tools for assessing the phenotypes and thus the functional role(s) of such microbes is still at a nascent stage (Liu *et al.*, 2006). Also, sheer numbers of organisms are a likely indicator of key roles they play in any ecological realm, thus isolation and study of the bacterial species present at the highest population levels (which we have deemed predominant) should be the point of departure in assessing the significance of rhizosphere community composition in relation to insect herbivory or herbicide application, for example.

## Results and discussion

There were large differences in the composition of the predominant Gram-negative rhizosphere bacteria based on the presence or absence of the root-attacking (as larvae) species *A. nigriscutis* and *A. lacertosa*, in each of the 3 years of this study (Figs. 1–4 and Tables 1–3). Particularly striking were the percent differences, based on the presence or absence of the insects, of such plant pathogenic species as *Pseudomonas syringae* van Hall, *Pseudomonas cichorii* (Swingle) Stapp and *Erwinia chrysanthemi*, Burkholder, all found either exclusively or with greater frequency in rhizospheres of *E. esula/virgata* with *Aphthona* spp. present.

Bacteria such as *Stenotrophomonas* spp. Palleroni and Bradbury 1993, *Pseudomonas chlororaphis* Guignard and Sauvageau 1894 and *Pantoea agglomerans* Gavini *et al.* 1989, with implications for possibly protecting the plant from the more lethal fungal root infections (which are operative as a key factor for rapid *E. esula/virgata* stand mortality), were present in both *Aphthona*-populated sites and sites lacking the flea beetle. However, S. *maltophilia* (Hugh, 1981) Palleroni and Bradbury 1993 was consistently found as a predominant species with much greater frequency in *Aphthona*-populated sites. These sites remained static in regard to stand density throughout the interval of the study (data not shown). Despite apparent insect damage-based stimulation of overall microbial biomass, *Stenotrophomonas* spp., well-known as antagonistic to plant pathogens, was most favored. There were also Gram-positive bacteria that have shown a capacity in combination with insects to reduce biomass of *E. esula/virgata* (Caesar and Kremer, 2008). Spurge infestations with both of these characteristics have persisted well after other infestations of *E. esula/virgata* have been dramatically reduced in density at TRNP. This may indicate that the complexity of the microbial community may contribute to pre-empting or antagonizing the insect/plant pathogen synergisms that cause more rapid stand reductions in biological control of this highly

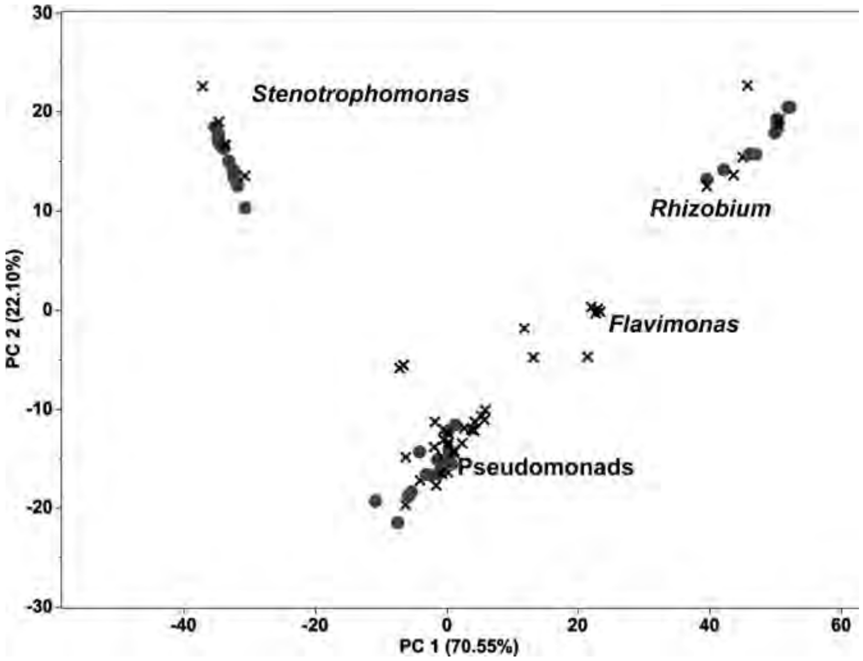


**Figure 1.** PCA of the rhizosphere community structure of predominant bacteria in relation to herbivory by *Aphthona* spp. Cumulative data from stands of four sites: (●) two sites with and (✕) two sites without the insect, 2001.

aggressive, deep-rooted perennial invasive plant. Pertaining to complexity, some studies have indicated that soil microbial complexity is associated with control of the soilborne plant pathogen *Rhizoctonia solani* (Garbeva *et al.*, 2006), and others have found no such association *per se* (Hiddink *et al.*, 2005). Neither study could identify specific components of the respective communities, so it is difficult to assess the underlying basis for the differing findings. Thus, our approach, although not as comprehensive as culture-independent methods of assessing the entire community nonetheless permits the identification of culturable species occurring

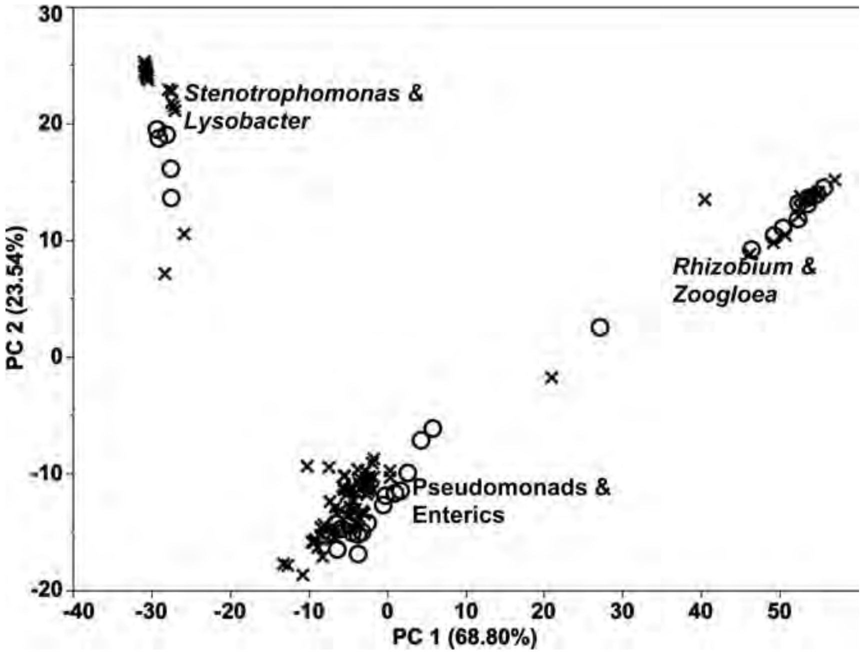


**Figure 2.** PCA of the rhizosphere community structure of predominant bacteria in relation to herbivory by *Aphthona* spp. Cumulative data from stands of four sites: (○) two sites with and (✕) two sites without the insect, 2002.

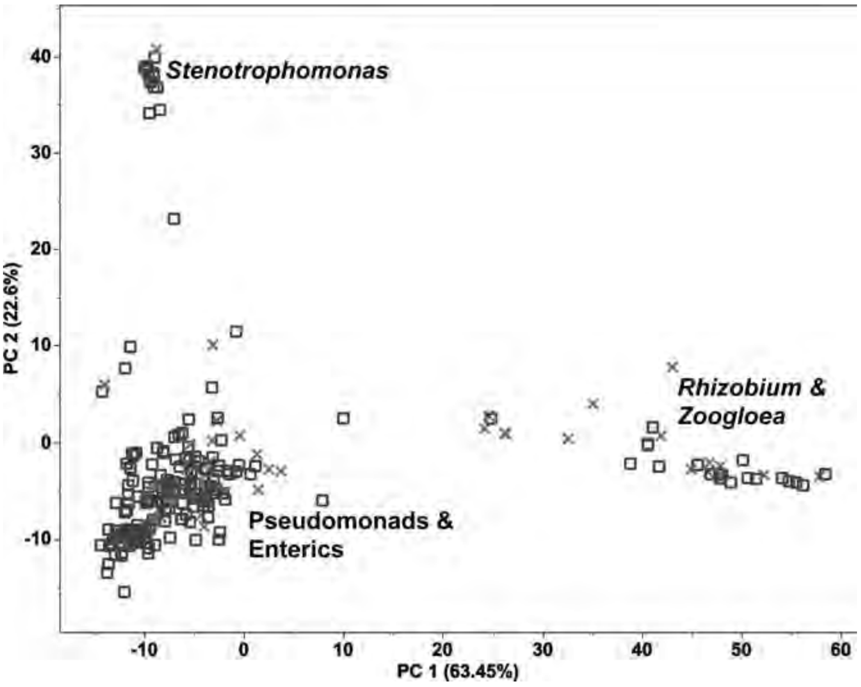


**Figure 3.** PCA of the rhizosphere community structure of predominant bacteria in relation to herbivory by *Aphthona* spp. Cumulative data from stands of four sites: (□) two sites with and (✕) two sites without the insect, 2003.

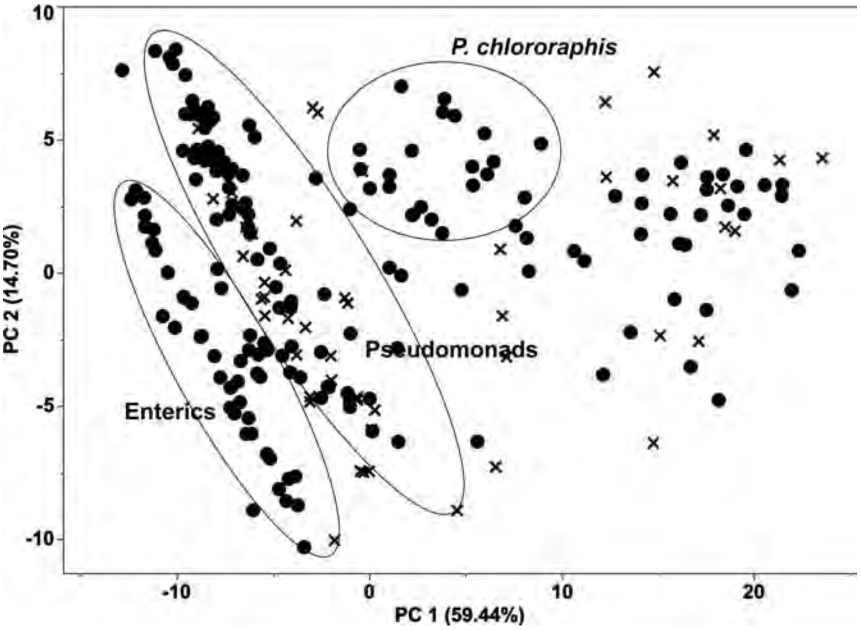


**Figure 4.** PCA of the rhizosphere community structure of predominant bacteria in relation to herbivory by *Aphthona* spp. Cumulative data from stands of four sites: (●) two sites with and (✕) two sites without the insect, 2003. The ellipses indicate pseudomonad and enteric groupings, wherein some groupings were nearly exclusive to rhizospheres of stands without *Aphthona* (enterics and *Pseudomonas chlororaphis*), whereas other groupings were mixed but with some evident phenotypic distinctions and differences in numbers (pseudomonads).

**Table 1.** Predominant microbial species from roots of leafy spurge at various sites with or without *Aphthona*, 2001.

| Microbial species | Percentage of isolates (of 81 isolates) | |
|---|---|---|
| | With *Aphthona* | Without *Aphthona* |
| *Pseudomonas putida* | 0 | 22.2 |
| *P. syringae* | 6.8 | 0 |
| *Pseudomonas fluorescens* | 20.5 | 13.3 |
| *P. cichorii* | 0 | 6.7 |
| *Flavimonas* spp. | 0 | 8.9 |
| *Stenotrophomonas* spp. | 38 | 6.6 |
| *Sphingomonas* spp. | 6.8 | 0 |
| *Rhizobium* spp. | 9.1 | 8.9 |
| *Zooglea* | 4.6 | 4.4 |

at the highest population levels in the rhizospheres of *E. esula/virgata*. We propose that complexity within such functional groups as those that contain pathogen-antagonistic or plant-beneficial strains, rather than overall diversity, may be more pertinent to such analyses. Previous studies have indicated that cultural methods track the results obtained through culture-independent methods (Garbeva *et al.*, 2006).

Work by others suggests that biological control insects themselves may not be the prime factors in biological control-based stand reductions (Larson and Grace, 2004), indicating that soil microbes have a great effect on whether biological control ultimately results in stand reductions or in a static state of target species density. The alterations in soil microbial community structure also have strong implications for the possibility of restoration of native plant communities. Analyses of Gram-positive rhizosphere bacterial communities, which contain isolates shown to cause 28–65% reductions in the biomass of *E. esula/virgata* (Caesar and Kremer, 2008) associated with *Aphthona* spp. herbivory were the subject of a companion study intended to be published separately.

**Table 2.** Predominant species from roots at various sites with and without *Aphthona*, 2002.

| Species | Percentage of isolates (of 121 isolates) | |
|---|---|---|
| | With *Aphthona* | Without *Aphthona* |
| ***Pseudomonas* spp.** | | |
| *P. syringae* | 17.3 | 0 |
| *P. cichorii/ viridiflava* | 4.8 | 0 |
| *P. fluorescens* | 12.5 | 15.6 |
| *P. putida* | 3.3 | 5.9 |
| *P. chlororaphis* | 1.9 | 0 |
| *P. huttiensis* | 1.9 | 6.3 |
| **Enterobacteria** | | |
| *Erwinia chrysanthemi* | 2.9 | 0 |
| *Pa. agglomerans* | 3.9 | 0 |
| *Other enterobacteria* | 0 | 6.3 |
| **Other** | | |
| *Stenotrophomonas* spp. | 19.2 | 6.3 |
| *Lysobacter enzymogenes* | 4.8 | 0 |
| *Rhizobium* spp. | 12.5 | 21.9 |
| *Variovorax paradoxus* | 0 | 15.6 |
| *Vibrio hollisiae* | 2.9 | 0 |

**Table 3.** Predominant species from roots at various sites with and without *Aphthona*, 2003.

| Species | Percentage of isolates (of 215 isolates) | |
|---|---|---|
| | With *Aphthona* | Without *Aphthona* |
| ***Pseudomonas* spp.** | | |
| *P. putida* | 21 | 34.5 |
| *P. chlororaphis* | 13.6 | 5.5 |
| *P. agarici* | 9.8 | 0 |
| *P. vancouverensis* | 2.7 | 7.3 |
| **Enterobacteria** | | |
| *Pa. agglomerans* | 13.6 | 1.8 |
| Other enteric species | 9.2 | 0 |
| **Other species** | | |
| *Stenotrophomonas* spp. | 7.6 | 1.8 |
| *Rhizobium* spp. | 1.3 | 1.3 |
| *Zoogloea* spp. | 5.6 | 7.3 |
| *Aquaspirillum autotrophicum* | 3.3 | 12.7 |

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# The endophyte-enemy release hypothesis: implications for classical biological control and plant invasions

**H.C. Evans**

*Summary*

Fungal endophytes are asymptomless colonizers of higher plants for all, or a part, of their life cycles. They range from latent pathogens to symbionts. There is increasing evidence that some form mutually beneficial, highly specialized or co-evolved associations with their hosts and that they provide the plant with an additional armoury to combat abiotic and biotic stresses, including pests and diseases. Thus, there may be a trade-off between reduced growth (in the short term), as nutrients are sequestered by the fungal mutualist, but increased overall long-term fitness as natural-enemy pressure is decreased. This tripartite balance may be lost when plants arrive in exotic ecosystems with incomplete guilds of both co-evolved endophytes and natural enemies. The enemy release hypothesis (ERH) explains why alien plants can become invasive. It is now hypothesized that another, more cryptic but still significant factor could also be involved: the presence or absence of mutualistic endophytes. Those neophytes arriving without co-evolved natural enemies but with mutualistic co-evolved endophytes would have a double advantage over local competitors. Such endophytic-enriched, alien-invasive weeds and those that form mutualistic associations with indigenous endophytes could help to explain the inconsistencies of some classical biological control introductions. Similarly, those alien plants that arrive and remain endophyte-free and without co-evolved natural enemies would have a distinct competitive advantage because they would have more resources to allocate to growth and reproduction, given, of course, that there are no significant pressures from indigenous natural enemies or that sufficient auto-defences are retained to overcome them. Such endophyte-depauperate alien-invasive weeds, however, remain highly susceptible to co-evolved natural enemies. This may explain the 'silver bullet' phenomenon, whereby the introduction of a single classical biological control agent can achieve complete control. This endophyte-enemy release hypothesis (E-ERH) is discussed with examples.

***Keywords***: coevolution, fungal mutualists, plant fitness.

## Introduction

There is now overwhelming evidence that all plants in natural ecosystems have developed symbiotic relationships with fungi (Rodriguez *et al.*, 2004) and that the mutualistic ones, especially those involving vesicular–arbuscular mycorrhizae (VAM), are ancient in origin (Brundrett, 2002). Indeed, it has been suggested that such associations were pivotal to the colonization of land by plants (Simon *et al.*, 1993; Blackwell, 2000; Brundrett, 2002). However, this mycotrophic theory had been discussed much earlier by Pirozynski and Malloch (1975), who hypothesized that the evolution of plants was made possible only through mutualistic partnerships with fungi, which enabled them to survive the stresses of life on dry land, where water and nutrients were the main constraints to colonization: 'Once an endosymbiotic relationship of a fungus with an alga was achieved, a blueprint for a terrestrial plant was drawn' (Pirozynski and Malloch, 1975).

Here, the form and function of mutualistic endophytic fungi is reassessed in the light of recent studies, and their possible significance in plant ecology is explored, leading to the hypothesis that their presence or absence may explain, at least in part, why some alien plants become invasive and why classical biological control can be so unpredictable as a management strategy.

## Definitions and concepts

There has been considerable debate and controversy as to the correct usage of the term *endophyte*, origi-

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nally coined by the founder of modern mycology, Heinrich de Bary in 1866 (Wilson, 1995). The ambiguities and confusion have been such (Wilson, 1993, 1995; Wennstrom, 1994) that it has been recommended the term should be defined according to context (Kirk *et al.*, 2001). Here, and specifically in relation to the proposed hypothesis, the use is restricted to fungi that invade living plants and colonize them without causing visible or immediate symptoms. Mycorrhizal fungi are excluded because, as dual plant–soil inhabitants, they are restricted to root systems in which there is synchronized plant–fungus development with nutrient transfer at specialized interfaces (Schulz and Boyle, 2005). In contrast, endophytic fungi lack the means of acquiring nutrients from soil but have evolved mechanisms that enable them to survive and live asymptomatically, at least initially, within the roots, stems and leaves of healthy plants (Brundrett, 2002).

Fungal endophyte associations with their host plants have been described as a continuum (Saikkonen *et al.*, 1998; Schulz and Boyle, 2005), ranging from parasitism (amensalism) through commensalism to mutualism (Lewis, 1985). This paper concentrates on the mutualists, those that form intimate associations with their plant hosts that are beneficial to both, providing protection from environmental stresses and microbial competition, as well as nutrients, for the fungus and increased resistance or tolerance to both abiotic and biotic stresses for the plant.

## Background to the hypothesis

Mutualistic endophytic fungi offer a variety of potential benefits to their host plants including growth enhancement, tolerance to abiotic factors (including drought, heat and heavy metals) and resistance to pests and diseases (Redman *et al.*, 2001; Rudgers *et al.*, 2004; Schulz and Boyle, 2005). The mechanisms involved may be wide-ranging and complex, the result of an ancient association (coevolution). In the case of conferring protection against plant pathogens, for example, these could range from antagonism, mycoparasitism, competitive displacement, to induced resistance (Evans *et al.*, 2003). It has now been established that anti-fungal, as well as anti-herbivore, secondary metabolites are produced by many endophytes (Latch, 1993; Christensen, 1996; Clay, 1997; Schardl and Phillips, 1997; Stone *et al.*, 2000). In addition, some produce novel growth-enhancement compounds (Varma *et al.*, 1999), whereas endophyte-free plants have been shown to activate defence mechanisms much more slowly than those with mutualistic endophytes (Rodriguez *et al.*, 2004), suggesting that they are also involved in inducing host resistance to pests and diseases. Finally, more recent studies have revealed unique trophic interrelationships between endophytes and their plant hosts, which enhance tolerance to both drought and heat (Rodriguez and Redman, 2005; Marquez *et al.*, 2007).

Most evidence for this increased fitness comes from studies of forage and turf grasses, particularly from plant associations in the subfamily *Pooideae*, with balansiaceous fungi belonging to the genus *Neotyphodium* (*Clavicipitaceae*: *Hypocreales*) because of their ecological and economic importance (Schardl and Phillips, 1997; Clay and Schardl, 2002; Bouton and Hopkins, 2003). *Neotyphodium* is a genus of highly specialized or obligate endophytic species that live systemically and intercellularly in all the aerial parts of their hosts and that are transmitted vertically in the grass seeds. There is increasing evidence, however, that similar benefits and increased plant fitness are also conferred to both non-woody and woody dicot hosts by horizontally transmitted, facultative endophytic fungi (Narisawa *et al.*, 2000; Wilson, 2000; Arnold *et al.*, 2003; Clay, 2004).

Obviously, there is a price to pay by the host plant for harbouring beneficial endophytes; although, as yet, quantitative data are lacking. Nevertheless, there is circumstantial evidence from field trials with turf grasses (*Poa* spp.) that shows that there is a significant cost involved because endophyte-free plants were notably more vigorous early in the season than those inoculated with endophytes. In contrast, later in the season, those with endophytes had markedly outperformed the plants lacking endophytes, as pests, diseases and drought stress took their toll (author, Rutgers University Experimental Station, 2001, personal observation). Moreover, there is indirect evidence of trade-offs in mycorrhizal fungi, where the carbon costs to the plant of supporting these mutualists have been found to be significant (Douds *et al.*, 1988).

### The endophyte-enemy release hypothesis

Plants in their centres of origin live in mutualistic relationships with a guild of specialized or co-evolved endophytic fungi that increase their tolerance of, or resistance to, both abiotic and biotic pressures, including co-evolved natural enemies. This protection comes at a price, with a trade-off in plant resources. Alien plants, especially dicot hosts, arriving in exotic ecosystems would have a depauperate endophytic mycobiota, freeing up resources for increased growth and reproduction. This, together with the absence of co-evolved natural enemies (enemy release hypothesis, ERH; Keane and Crawley, 2002), would enhance significantly their fitness. Given that these endophyte-free aliens have sufficient auto-defence mechanisms to overcome the pressure from indigenous natural enemies, they then would have increased competitive advantage. The result would be a dominance of these enhanced or favoured species that would increase over successive generations. Thus, neophytes with weedy traits would tend to become dominant and invasive. However, such plants would be highly vulnerable to co-evolved natural enemies. This could explain the phenomenon of the

'silver bullet' in classical biological control, whereby the introduction of a single biological control agent can successfully and often unexpectedly, bring about the complete control of a rampant, invasive alien weed. Other alien plants, especially grasses with vertically transmitted endophytes, may arrive with their mutualistic endophytes, which, in the absence of co-evolved natural enemies, would give them a double advantage over local competitors. Such endophyte-enriched, alien-invasive weeds and those forming mutualistic associations with indigenous endophytes that afford protection from pests and diseases could help to explain why some classical biological control introductions fail to live up to expectations or that have only limited impact on the target weed.

The endophyte-enemy release hypothesis (E-ERH) could help to resolve the on-going debate on the validity of the ERH (Wolfe, 2002; Mitchell and Power, 2003; Colautti *et al.*, 2004; Parker *et al.*, 2006), as well as clarify inconsistencies in both the new encounter and the evolution of increased competitive ability hypotheses (Hokkanen and Pimentel, 1984; Blossey and Notzold, 1995). It also has resonance with the recently proposed resource-ERH (Blumenthal, 2006), with a possible parallel situation in animal invasions, if protective endophytes can be compared to or are analogous with animal immune defence systems (Lee and Klasing, 2004).

# Evidence for the hypothesis

## Monocot hosts

Evidence for specialized or co-evolved mutualistic associations is unequivocal in the grass–*Neotyphodium* systems (Schardl and Phillips, 1997; Schardl and Moon, 2003). There is no clearer demonstration of the ecological and practical importance of mutualistic endophytes than the *Poa annua–Neotyphodium* association in northern United States, where endophtye-enriched seed is now routinely supplied to the turf-grass industry (Bouton and Hopkins, 2003). The serendipitous discovery of the co-evolved endophyte in seed of *P. annua* L. imported from northern Europe (the centre of diversity), as part of a breeding programme, led to research that demonstrated that the fungus not only afforded protection against generalist herbivores and pathogens but also conferred drought tolerance (J.F. White, Rutgers University, personal communication, 2002). Tests showed, however, that this fungus is not infective to *Poa pratensis* L. (Kentucky blue grass, actually a European species from the Mediterranean region), which is in high demand as a turf grass in southern United States. Ecological and economic logic dictate that surveys in southern Europe would pay dividends.

In another example, which at first sight, may appear to contradict the E-ERH, involves *Lolium* (*Festuca*) *arundinaceum* (Schreber) S.B. Darbyshire or tall fescue and its co-evolved endophyte, *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin. Tall fescue is a European species of high agronomic importance in North America despite the presence of the endophyte that produces highly toxic ergot alkaloids (Cross, 2003). Endophyte-infected plants are more vigorous, drought-tolerant and resistant to herbivores than endophyte-free ones, and one cultivar in particular (Kentucky 31), with enhanced endophyte activity, has now become a major invader of natural communities where it impacts directly on the native flora and fauna with long-term effects on successional dynamics and food webs (Clay and Holah, 1999). It has been argued that this is evidence of ecosystem vulnerability to human-induced invasion by an inbred, highly competitive exotic species (Saikkonen, 2000; Saikkonen *et al.*, 2006) rather than a natural model. Whatever the interpretation, indirectly it lends support to the E-ERH, demonstrating the ecological importance of co-evolved, mutualistic endophytes and the invasive threat from such associations in the absence of co-evolved natural enemies. Should classical biological control ever be considered as a management strategy for this invasive grass, the result would be an arms race between the endophyte and any introduced (co-evolved) natural enemies. This example also begs the question: do similar endophyte associations also occur in the invasive African grasses currently threatening the long-term stability not only of the Amazon region but also of global weather patterns (Mack *et al.*, 2000)?

## Dicot hosts

In sharp contrast, it is much less likely that co-evolved endophytes will be carried to new ecosystems with their dicot hosts, given that these are horizontally transmitted and that most introductions (accidental or deliberate) are from seed. In effect, it would be a similar situation to that of co-evolved natural enemies, where there are few examples of them arriving together with their weed hosts. Therefore, it would be expected that most invasive alien dicots lack specialist or co-evolved endophytes.

The degree of specificity of dicot endophytes, however, is not as clear-cut as for the grass endophytes discussed earlier, and it is probable that these are facultative rather than obligate in that, unlike *Neotyphodium*, they can survive saprophytically (Wilson, 2000). Surveys for endophytes of cocoa (*Theobroma cacao* L.) and its relatives in their South American centres of origin revealed that the stems and pods of healthy wild trees have a rich and unique endophytic mycobiota that becomes depauperate in plantation trees in exotic situations (Evans *et al.*, 2003; Crozier *et al.*, 2006). *In vitro* studies to test their biological control potential further demonstrated that some of these novel endophytes, pertaining to the *Clavicipitaceae* and *Hypocreaceae* (*Hypocreales*), are highly antagonistic to the co-evolved fungal pathogens of cocoa and, in addition,

produce secondary metabolites known to be involved in plant defence mechanisms (Holmes *et al.*, 2004; Samuels *et al.*, 2006).

From this work, there is an indication that specialized, perhaps co-evolved, endophytes dominate in native habitats, but these are replaced by generalists when the host is moved to exotic ecosystems. Similar results have been reported for other woody plant hosts (Wilson, 2000), and further support is coming from ongoing surveys of the endophytes associated with *Lantana camara* L. in both natural and degraded habitats in Brazil, where this plant is indigenous, as well as in its exotic invasive range in Pakistan (author, unpublished results). The endophytes isolated from *L. camara* in degraded sites in Brazil showed similarities with those recorded from Pakistan in that these belonged predominantly to a few well-known generalist fungal genera (*Glomerella/Colletotrichum, Phomopsis*), whereas those from a forest site population comprised an extremely rich mycobiota with many unusual genera being represented. It is tempting to suggest that these are part of a specialized endophytic guild of fungi that form mutualistic associations with *L. camara* and that, like the co-evolved natural enemies, they have been left behind as the plant host has been moved around the world.

Preliminary studies on Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decr., are yielding similar results. This plant, in urban situations in the United Kingdom, is virtually free of endophytes, whereas in climax habitats in Japan, it has a rich and diverse endophytic mycobiota (H. Evans, unpublished results). So much so that contaminating endophytes have hampered the culture and study of the fungal component of the plant's co-evolved natural enemies. One of these, belonging to a monotypic asexual genus, which, unusually, also produces its sexual stage (a new discomycete genus) in culture, can be reinoculated into and readily reisolated from healthy knotweed leaves. Whether this species and the other endophytes from Japan are specific or co-evolved mutualists remains to be proven. However, it is evident that sophisticated recognition mechanisms are involved, enabling the fungus to bypass the plant's defences.

## Classical biological control

The 'silver bullet' examples in classical biological control could be explained, at least in part, on the basis of the invasive alien weed having lost its protective co-evolved endophytes and not acquiring indigenous generalist mutualists to fill this role. Thus, the fitness of any introduced co-evolved natural enemy would be increased accordingly. An analysis of the successful rubber vine project in Australia offers empirical supporting evidence. This Madagascan asclepiad, *Cryptostegia grandiflora* (Roxb. ex R. Br.) R. Br., which covered more than 40,000 $km^2$ of northern Queensland, has now been stopped in its tracks after the release of a co-evolved rust, *Maravalia cryptostegiae* (Cummins) Y. Ono (Tomley and Evans, 2004). Unexpectedly, host mortality has been exceptionally high (75%) because of a lethal combination of rust- and drought-induced stress, whereas pod set and seedling recruitment have been almost nonexistent.

Such dramatic impacts and high mortality are atypical of obligate pathogens especially in natural ecosystems and was never observed in Madagascar, where the rust constitutes part of a guild of natural enemies keeping the rubber vine population in check but neither eliminating flowering and fruiting nor killing seedlings and mature plants.

# Discussion

The E-ERH is just one amongst a plethora of hypotheses put forward to explain invasiveness by alien species, especially by plants. Impressively, Colautti *et al.* (2004) list no less than eight nonexclusive theories for invasion success. Others have been added since (Müller-Schärer *et al.*, 2004; Blumenthal, 2006). This has led to confusion and controversy, not to say a heady mix of acronyms.

Each hypothesis could in itself explain a part of invasion ecology, or more likely, each invasive weed needs to be dealt with on a case-by-case basis. Clearly, the successful 'silver bullet' classical biological control projects against invasive weeds must have been driven by the ERH. In these cases, the E-ERH may further explain why the release of a single natural enemy can have such a dramatic and profound impact. Conversely, the presence of co-evolved mutualists, in the absence of co-evolved natural enemies, offers an explanation as to why certain grasses have become major invasive species with the ability to alter plant communities and reduce biodiversity (Clay and Holah, 1999; Mack *et al.*, 2000). The E-ERH has direct pragmatic implications perhaps more so in plant disease rather than weed-invasion ecology. In fact, the germ of the E-ERH was sown during a project to evaluate the biological control potential of co-evolved endophytes in the centres of origin of the two major diseases of *T. cacao*. The finding of unique endophytes in wild cocoa with demonstrable antagonism towards the cocoa pathogens, together with their absence in cultivated cocoa, points to a role for mutualistic endophytes in plant protection (Evans *et al.*, 2003; Holmes *et al.*, 2004; Samuels *et al.*, 2006). In the future, there could be the intriguing possibility that crop plants, like turf grasses in the United States, will be marketed as 'endophyte-enriched', planting material being inoculated with co-evolved mutualists to protect not only against pests and diseases but also against abiotic stresses, notably drought. Such inoculations with mycorrhizal fungi are now standard practice in tree nurseries.

Perhaps it is fitting to touch on the subject of mycorrhizae because they may also play a role in invasion biology. Indeed, mutualistic ectomycorrhizal (EM) fungi

offer an explanation as to why exotic pine species have increased fitness and why, in some ecosystems, they have become highly invasive (Richardson *et al.*, 2000). In contrast, non-specific VAM fungi (*Glomales*) occur in all soils and, seemingly, would readily be acquired by indigenous and non-indigenous plants alike (Read, 1999). They have even been considered to be 'arguably the most important group of all living organisms' (Brundrett, 2002). Significantly, however, there are plant families that are predominantly non-mycorrhizal, including *Amaranthaceae, Brassicaceae, Chenopodiaceae, Commelinaceae, Cyperaceae, Polygonaceae* and *Urticaceae*. Many of these 'are pioneer colonizers of marginal habitats or weedy, opportunistic invaders of disturbed soil', that 'have expanded into more marginal environments since mid-Mesozoic, a trend which appears to be accelerated by man's escalating agricultural and industrial activities' (Pirozynski, 1981). Could it be that they no longer needed VAM fungi and the trade-offs this entailed, relying instead on mutualistic endophytes for competitive advantages? Indeed, the main feature of the roots of these plant families is the capacity to actively exclude VAM fungi through the release of anti-fungal metabolites (Brundrett, 2002). The exclusion of VAM fungi would conserve energy, increase fitness and, therefore, should be another factor to be included in the long list of why some plants become invasive.

Tantalizingly, Richardson *et al.* (2000) briefly reflect on fungal endophytes as possible promoters of plant invasions, concluding that: 'the specificity and the nature of such associations are poorly known as is their role in invasion'. Here, it is proposed that their role, in the case of specialized or co-evolved mutualistic endophytes, is twofold: their presence increasing plant fitness in the absence of co-evolved natural enemies, especially in grass hosts with vertically transmitted endophytes; their absence coupled with release from co-evolved natural enemies, contributing to increased plant fitness, especially in dicot hosts with horizontally transmitted endophytes, but leaving them highly vulnerable to classical biological control agents.

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# Multiple-species introductions of biological control agents against weeds: look before you leap

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*Summary*

Biological control practitioners have frequently debated the issues behind single vs multiple species introductions against target weeds. In the case of weed biological control, conventional wisdom is that multiple species should be used on the assumption that several species are more likely to have a greater controlling impact than a single species alone. This debate is rehearsed with reference to the biological control of four species of Australian acacias in South Africa: long-leaved wattle (*Acacia longifolia* (Andr.) Willd.), golden wattle (*Acacia pycnantha* Benth.), Port Jackson willow (*Acacia saligna* (Labill.) H. Wendl.) and rooikrans (*Acacia cyclops* A. Cunn. ex G. Don), where the impacts of both gall-forming and seed-reducing agents were intended to be additive and possibly synergistic. Evaluation and observations of these specific cases show that multiple-species introductions can be beneficial, but in at least one case (*A. cyclops*), the wisdom of these releases is questionable and potentially even detrimental. This suggests the need for extreme caution when planning multiple-species introductions against a target weed species.

***Keywords***: multiple species, *Acacia*, biological control.

## Introduction

For many years, biological control practitioners have discussed and debated the merits of releasing multiple as opposed to single species of biological control agents in weed control programmes. The focus of such discussion has been multifaceted, either in terms of the effectiveness of the actual control (Myers, 1985; Myers *et al.*, 1989; Story *et al.*, 1991; Müller-Schärer and Schroeder, 1993; Hoffmann and Moran, 1998; Anderson *et al.*, 2000), competitive interactions between agents (Zwölfer, 1973; Ehler and Hall, 1982; Denno *et al.*, 1995; Woodburn, 1996; Briese, 1997; McEvoy and Coombs, 2000), the best timing or sequence in which to introduce agents (Briese, 1991; Syrett *et al.*, 1996), or in terms of risk, safety and direct and indirect non-target effects (Myers, 1985; Simberloff and Stiling, 1996; Callaway *et al.*, 1999; Denoth *et al.*, 2002; Pearson and Callaway, 2005).

For most weed biological control projects, the highest levels of 'success' have been achieved using multiple agents, either because there has been a cumulative or synergistic effect of all agents working together (e.g. Hoffmann and Moran, 1998) or because as agent numbers are increased, there is likely to be a greater probability that the most suitable species will be released, often with a single agent being responsible for the success (Myers, 1985). Alternatively, the introduction of more agents may ultimately provide a higher probability of biological control over wider geographical ranges, due to different agent species performing better under different conditions (DeBach, 1964; Baars and Heystek, 2003; Day *et al.*, 2003). In many cases, new and additional agents are released prematurely, either because existing agents have not been adequately evaluated or because agents have not been provided an opportunity to achieve their full potential (McFadyen, 1998; McEvoy and Coombs, 2000). Unfortunately, predicting the effectiveness of, and possible interactions between, potential biological control agents remains an

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ongoing and daunting challenge (Cullen, 1995; Zalucki and van Klinken, 2006).

The biological control programmes against four invasive Australian *Acacia* species in South Africa are discussed with respect to these issues. They demonstrate that although multiple-agent releases are usually beneficial, there are times when this may not be the case and releases of more than one agent should be planned with caution.

## Biological control of *Acacia* species in South Africa

During the last 30 years, biological control has been implemented against nine of the most invasive Australian *Acacia* species in South Africa (Dennill *et al.*, 1999). Collectively, these programmes have largely been governed by conflicts of interest over desires to control the plants whilst continuing to exploit them commercially for production of tannin, for timber and pulp, for fire wood and for dune binding. Consequently, the choice of biological control agents has been restricted, for the most part, to agents that limit the reproductive output of their hosts, thereby reducing invasiveness but not the useful attributes of the plants. Four of these acacias have been subject to control by two agent species released sequentially (Table 1), and they are the subject of discussion here.

***Acacia longifolia* (Andr.) Willd. (long-leaved wattle):** The gall-forming wasp, *Trichilogaster acaciaelongifoliae* Froggatt (Hymenoptera: Pteromalidae), was released on *A. longifolia* in South Africa during 1982 (Dennill and Donnelly, 1991). The wasps dispersed readily and reduced seed production on *A. longifolia* by more than 95%, even causing some suppression of vegetative growth of the plants (Dennill, 1988). However, there were two situations where *T. acaciaelongifoliae* was not fully effective: (a) in the hot, arid, inland areas and in the elevated, moist, mist-belt regions of the country where climatic conditions curb population expansion of the wasps (Dennill and Gordon, 1990) and (b) *A. longifolia* plants growing close to rivers do not suffer water stress and still produce substantial seed loads despite high levels of galling by the wasp (Dennill *et al.*, 1999).

Although the impact of *T. acaciaelongifoliae* was being studied, a second agent, a seed-feeding weevil, *Melanterius ventralis* Lea (Coleoptera: Curculionidae), had been proposed for control of *A. longifolia* and was being tested in quarantine. By 1985, the need for an additional agent was deemed to be necessary, and the first releases of *M. ventralis* were made. The seed-feeding weevils established readily at release sites and have subsequently played an important supplementary role in the suppression of seed production by *A. longifolia* (Dennill *et al.*, 1999; Donnelly and Hoffmann, 2004).

***Acacia pycnantha* Benth. (golden wattle):** Following the success of *T. acaciaelongifoliae* on *A. longifolia*, a related species of gall-forming wasp, *Trichilogaster signiventris* (Girault) (Hymenoptera: Pteromalidae), was released against *A. pycnantha* during 1987. After a slow start, when it was believed that the wrong strain of *T. signiventris* may have been imported (Dennill and Gordon, 1991), and additional releases in 1992, levels of galling increased dramatically, and the insects became abundant throughout the range of *A. pycnantha* by 1998. Besides substantial reductions in seed production due to the wasps, in some cases, extensive galling caused collapse of branches and toppling of whole trees (Dennill *et al.*, 1999; Hoffmann *et al.*, 2002). Although initial indications were that no additional agents would be required to further reduce seed production, monitoring of pod and gall loads (in 2004 and 2005) demonstrated that many seed pods were still being produced despite the damage caused by *T. signiventris*.

The successful combination of the gall former and a seed feeder in the *A. longifolia* programme paved the way for a similar approach with *A. pycnantha*, and in 2005, the seed-feeding weevil, *Melanterius maculatus* Lea (Coleoptera: Curculionidae), was released. Although it is still too early to draw conclusions regarding the combined impact of the two agents, indications are that both agents will complement each other in reducing seed loads of *A. pycnantha* plants as is the case on *A. longifolia*.

***Acacia saligna* (Labill.) H. Wendl. (Port Jackson willow):** Biological control of *A. saligna* had been recommended as a priority from the outset of the programme against the Australian acacias (Neser and Annecke, 1973). The gall-forming rust fungus, *Uromycladium tepperianum* (Sacc.) McAlp. (Urediniales: Raveneliaceae), was selected as being a suitably damaging agent in that it could reduce reproductive output and also weaken the plants and ultimately cause their death (van den Berg, 1977). After its release in 1987, *U. tepperianum* rapidly dispersed throughout the range of *A. saligna*. Long-term evaluation studies demonstrated that the rust was an extremely effective agent, reducing population densities of adult trees by up to 85% (Wood and Morris, 2007). However, as in the case of *A. longifolia* and *A. pycnantha*, *A. saligna* was still able to produce large seed loads before succumbing to the effects of high levels of galling.

Again, the need was recognized for a second agent to target the remaining seeds, and another seed-feeding weevil, *Melanterius compactus* Lea (Coleoptera: Curculionidae), was released against *A. saligna* in 2001. Although the introduction of *M. compactus* is relatively recent, preliminary monitoring indicates that, like its counterpart on *A. longifolia*, the weevils are playing an important supplementary role in curbing the production of viable seeds on *A. saligna*.

***Acacia cyclops* A. Cunn. ex G. Don (rooikrans):** *A. cyclops* was the last of the four species under discussion to be subjected to biological control. In the early 1990s, there was a strong focus on the *Melanterius*

group of weevils, which were readily available and easy to collect and had been shown to be sufficiently host-specific and damaging to warrant consideration (Impson and Moran, 2004). In 1991, the first release of *Melanterius servulus* (Pascoe) (Coleoptera: Curculionidae) was carried out, followed in 1993 by more widespread releases. Although the weevils established successfully, they were relatively slow to build up their populations, and dispersal was also limited (Impson *et al.*, 2004; Impson, 2005). Despite this, levels of seed damage increased with time at many of the release sites, with up to 95% seed damage being recorded within 5 years of release at some of the sites. Manual redistribution has been used to compensate for slow rates of natural dispersal.

In 2001, a proposal was made that a second agent, a flower-galling midge, *Dasineura dielsi* Rübsaamen (Diptera: Cecidomyiidae), should be released to supplement the activities of *M. servulus*. It was anticipated that the midge would fulfill a complementary role and have good dispersal abilities, which would thus compensate for the problem of slow dispersal rates of the weevil. At the time, some concerns were expressed regarding possible competitive interactions between the two control agents (i.e. by galling the flowers, the midge would indirectly remove the food source of the weevils), but the matter of containing large invasions of *A. cyclops* was considered a priority and additional restrictive measures against this plant were strongly supported.

Following the establishment of *D. dielsi*, the midge dispersed extremely rapidly (hundreds of kilometers per year) throughout the range of *A. cyclops* (J. Moore, personal communication, 2003), and with its multivoltine life cycle, populations of the midge exploded. It initially appeared that the proverbial 'silver bullet' had been released, and *A. cyclops* trees had been all but sterilized by the extremely high levels of galling. However, this situation did not persist, and midge populations have become less stable, resulting in considerable variation in the amount of pod set between sites and between years (F. Impson, C. Kleinjan and J. Moore, unpublished results). This has obvious implications for *M. servulus* because the weevils may no longer be able to sustain their populations when faced with an unpredictable food source, and ultimately, the success of the biological control programme against *A. cyclops* may be compromised.

## Discussion

In these four cases of biological control against imported Australian acacias, there was a clear rationale, based on available knowledge, which governed the pattern and sequence of the releases of agents (Table 1), and in each case, the release of two agents has been justified.

For each of *A. longifolia*, *A. pycnantha* and *A. saligna*, a gall-forming agent was released before being followed up by a seed-destroying weevil (Table 1). In all of these programmes, the sequence of releases (i.e. a gall former preceding a seed feeder) was largely determined by opportunistic and pragmatic considerations. Agents that were readily available, obviously damaging to the host plant, abundant and easy to collect and amenable to specificity testing enjoyed priority. In the case of *A. longifolia*, the release of two species of agents occurred within 3 years of each other, and it is possible that if practical circumstances had been different the order of release could have been reversed. The cases of *A. pycnantha* and *A. saligna*, respectively, are different in the sense that considerable time elapsed between the releases of the first and second agents. The reason for this was a conscious decision to evaluate the impact of the gall formers acting on their own, before taking the decision to release a supplementary agent. In both cases, events were to prove that although the gall formers were highly effective, there were more than sufficient seeds left in the system to maintain populations of the host plants at problematic levels. There was a clear need for the seed-feeding weevils to reduce the numbers of viable seeds.

The pattern for *A. cyclops*, however, is different in that a seed-feeding weevil species was released first, followed several years later by the release of a gall midge. Again the sequence of release was determined by pragmatic and opportunistic circumstances and was influenced by strong demands for additional control measures against *A. cyclops*, particularly in view of the slow dispersal rates of *M. servulus*. The, gall midge, *D. dielsi*, was not an obvious choice of agent, primarily because of doubts about the effectiveness of gall midges as biological control agents (Goeden and Louda, 1976; McFadyen, 1985; Wehling and Piper, 1988; Carlson and Mundal, 1990; Harris and Shorthouse, 1996) and because from the outset there were some concerns over a potential conflict with *M. servulus*. Eight years elapsed before it was decided that a supplementary agent was needed.

The *A. cyclops* programme differs from the others in one other important respect. In the case of *A. longifolia*, *A. pycnantha* and *A. saligna*, the gall-forming agents are essentially univoltine, exert pressure on the plants and substantially reduce seed production, but in most circumstances, there are sufficient seeds remaining locally or in a wider area to sustain populations of the seed-feeding weevils. In other words, the evidence suggests that the effects of the agents are complementary. The gall-forming cecidomyiid, *D. dielsi*, on *A. cyclops*, by contrast, goes through several generations a year, most of which coincide with the peak flowering period of the plant (the females lay their eggs in the flowers), which initially led to enormous gall loads and the virtual or complete elimination of pods at sites. Subsequently, levels of pod production have been extremely variable. Of concern is the possibility that the fluctuations in pod set will destabilize populations of *M. servulus* and render the beetles unable to exploit and

**Table 1.** The four species of Australian acacias targeted for biological control in South Africa, using in each case sequential releases of two agent species, all imported from Australia. In certain cases (marked by asterisk), there were previous releases, but they were unsuccessful.

| *Acacia* species (Mimosaceae) | Agent released | Date of first release | Release interval between agents (years) | Mode of action | References |
|---|---|---|---|---|---|
| A. *A. longifolia* (long-leaved wattle) | 1. *Trichilogaster acaciaelongifoliae* | 1982 | 3 | Induces extensive gall formation | Dennill, 1988; Dennill and Donnelly, 1991 |
| | 2. *Melanterius ventralis* | 1985 | | Destroys seed | Dennill and Donnelly, 1991 |
| B. *A. pycnantha* (golden wattle) | 1. *T. signiventris* | 1992* | 13 | Induces extensive gall formation | Dennill and Gordon, 1991; *Dennill *et al.*, 1999 |
| | 2. *M. maculatus* | 2005 | | Destroys seed | F. Impson, unpublished results |
| C. *A. saligna* (Port Jackson willow) | 1. *Uromycladium tepperianum* | 1987 | 14 | Induces fungal galls on reproductive and vegetative tissue | Morris, 1991 |
| | 2. *M. compactus* | 2001 | | Destroys seed | F. Impson, unpublished results |
| D. *A. cyclops* (rooikrans) | 1. *M. servulus* | 1993* | 8 | Destroys seed | *Dennill *et al.*, 1999; Impson, 2005 |
| | 2. *Dasineura dielsi* | 2001 | | Induces galling of flowers | Adair, 2004 |

destroy the surfeit of seeds that develop when *D. dielsi* is less effective. At times when seeds are scarce, the situation is further exacerbated by rodent and bird predation of the seeds. The adult weevils also feed widely on the ripening seeds, leaving virtually no seeds that are in a suitable condition for oviposition. Under these conditions of extreme seed scarcity, the weevil populations are in danger of becoming extinct locally or even over wide areas.

It is still too early to predict the long-term outcome of this programme. Preliminary studies indicate that the unstable conditions over the last few years have impacted on *M. servulus* populations at some monitoring sites, and if this situation persists, it may prove to be inimical to the biological control programme against *A. cyclops* in the long term. Alternatively, midge populations may ultimately stabilize at levels where sufficient pods are consistently available at sites. Under such conditions, it is anticipated that *M. servulus* populations would build up again and that the actions of *D. dielsi* and *M. servulus* could be additive, as it is for the other *Acacia* species with two agents. In addition, the objective of harnessing the high dispersal abilities of *D. dielsi* would also have been realized.

Apart from the fact that the introduction of organisms contains inherent risk, the broader ecological consequences of introductions have received little attention and remain poorly understood. Weed biological control is only contemplated in situations where mechanical and/or chemical control of invasive plants is impractical or prohibitively expensive. Predicting the outcome of introductions remains problematic because, frequently, the interacting attributes of the agent, the target weed and the environment are extremely complex. Furthermore, the introduction of each additional agent introduces another tier of complexity, complicating the ability to correctly predict outcomes.

In the case of the releases of a second agent onto *A. pycnantha* and *A. saligna*, sufficient time had elapsed between the introductions of the gall formers and the subsequent decision to release seed feeders; the impacts of the gall formers were well understood, and a clear need for an additional agent that would target residual seed production was identified. In addition, extensive knowledge of the attributes of *Melanterius* spp. and their potential as biological control agents in South Africa was available. With *A. cyclops*, sufficient time had elapsed after the introduction of *M. servulus* for adequate evaluation of its performance and the recognition of its limitations. However, the ability to predict the outcome for the midge and its possible interactions with *M. servulus* was limited. The situation with *A. cyclops* in South Africa highlights the need for extreme caution when contemplating multiple species introductions and adds credence to the rule that biological control agents in any situation should only be introduced where circumstances demand and where the best predictions, as a result of experience, intuition or modeling, suggest that these multiple species introductions will not worsen the situation.

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# Clipping the butterfly bush's wings: defoliation studies to assess the likely impact of a folivorous weevil

**D.J. Kriticos,[1] M.S. Watt,[2] D. Whitehead,[3] S.F. Gous,[4] K.J. Potter[5] and B. Richardson[4]**

*Summary*

Predicting agent success is a topic that has attracted much attention from the biological control community. Although the likely success of agents establishing in a new environment remains elusively unpredictable, we can often gain an impression of the likely nature of the agent's impact in different environments should it establish in reasonable numbers. The butterfly bush, or buddleia (*Buddleja davidii* Franch.), is a major weed problem in many regions with temperate or Mediterranean climates and has been identified as the highest priority for biological control in Europe. In New Zealand, it has invaded disturbed sites such as plantation forest coups, roadsides, earth slips and gravel river beds. To combat buddleia in New Zealand, a biological control programme was commenced around 1990. Whilst host-specificity testing was being completed on *Cleopus japonicus* Wingelmüller, a leaf-feeding weevil, defoliation experiments were undertaken to assess its likely impact on the growth and survival of its prime host, buddleia. Seasonal defoliation studies revealed that in the absence of plant competition, buddleia was quite resilient and able to recover rapidly from severe defoliation. Experiments with plant competition, leaf consumption rates and insect developments rates were used to develop a model to explore the likely impact of *C. japonicus*.

***Keywords***: *Buddleja davidii*, *Cleopus japonicus*, compensatory growth, growth modelling, simulated herbivory.

## Introduction

Predicting the likely success of a biological control agent is a topic that has attracted much attention from the biological control community. The prime challenge for biological control practitioners after ensuring agent safety is to select agents that have a high probability of establishing and, if established, will have a significant negative impact on the target weed. The success of this endeavour depends partly on how well the effects of the agent on the growth and survival of the target weed species can be predicted in the country of release (McFadyen, 1998).

Although numerous examples of complete or partial control of weed species by biological control agents have been reported, there are also many instances where control of the target weed has been negligible (McEvoy *et al.*, 1991; Ooi, 1992; Hoffmann, 1995; McFadyen, 1998; Julien and Griffiths, 1999). Whilst predicting the success of individual agents in establishing in a new environment remains elusive, we may be able to at least gain an impression of the likely nature of the agent's impact in different environments should it establish in reasonable density. For agents that defoliate plants, it may be appropriate to undertake studies to gauge the impact of different defoliation regimes on various aspects of the plant's natural history.

A broad understanding of how attack by a biological control agent influences a weed's growth and life history traits is helpful for prioritizing guilds of insects or pathogens for inclusion in biological control programmes and quantifying the level of control that can be expected from individual agents (Kriticos, 2003;

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Kriticos *et al.*, 2003). Knowledge of the per capita impacts of putative agents and relative ranges of their natural rate of increase can provide practitioners with an indication of the likely relative impacts that agents with different modes of attack might have on the target plant (Raghu and Dhileepan, 2005).

For folivorous biological control agents, accurate determination of their influence on plant growth, and how these interactions change across environmental gradients, requires an understanding of the mechanisms by which leaf area reductions influence growth processes. In many species, reductions in biomass are proportionately lower than reductions in leaf area (Langstrom and Hellqvist, 1991; Lavigne *et al.*, 2001), as plants can respond to defoliation through compensatory growth (McNaughton, 1983; Strauss and Agrawal, 1999). Compensatory responses that have been observed include increased biomass allocation to leaves (Pinkard and Beadle 1998) and increases in photosynthetic activity (Heichel and Turner, 1983; von Caemmerer and Farquhar, 1984; Trumble *et al.*, 1993). Leaf tissue removal has also been shown to either increase (Mabry and Wayne, 1997) or reduce (Dirzo, 1984; Mabry and Wayne, 1997) longevity of remaining leaves.

The butterfly bush, or buddleia (*Buddleja davidii* Franch., Buddlejaceae), is a major weed problem in many regions with temperate or Mediterranean climates (Fig. 1a, b), and it has been identified as the number one priority for biological control in Europe (Sheppard *et al.*, 2006). *Cleopus japonicus* Wingelmüller (Coleoptera: Curculionidae) is a leaf-feeding weevil that has been identified as a biological control agent for buddleia. After extensive host-specificity testing, *C. japonicus* was released in New Zealand in late 2006. Initial results indicate that it appears to be establishing well in the field, although the field populations are yet to experience a winter in New Zealand. Before releasing this agent, we undertook a study to assess the potential impact of defoliation and improve biological control practice. The method outlined in this paper provides

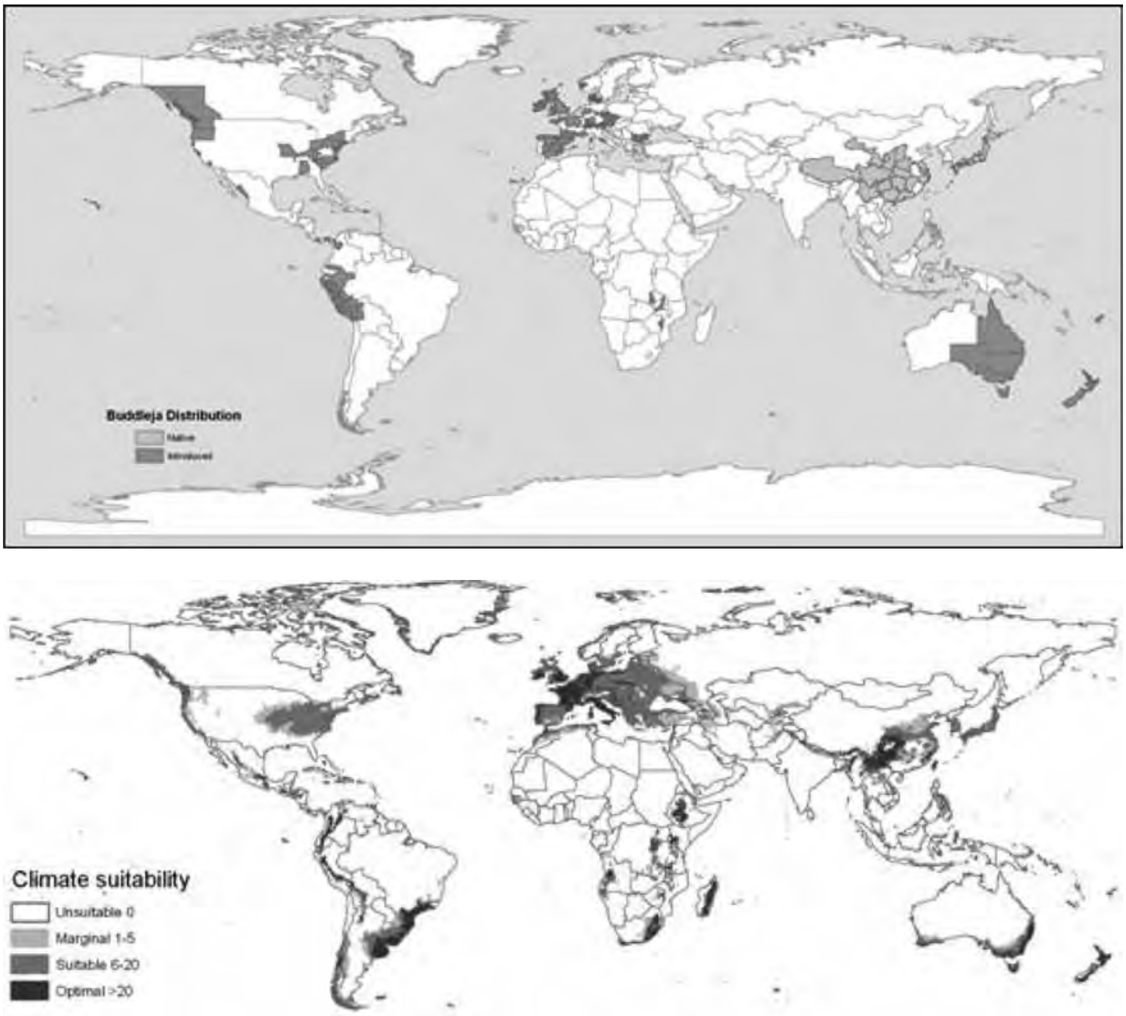


**Figure 1.** The global distribution of *Buddleja davidii.* (a) The known distribution and (b) the climatic suitability (potential distribution) modelled using CLIMEX (D.J. Kriticos, K.J. Potter and N. Alexander, 2005, unpublished internal report 37986, Ensis, Rotorua, New Zealand).

a framework for quantifying the net growth impact of feeding by folivorous biological control agents on weeds. This method also provides a means of understanding critical levels of defoliation needed to achieve target levels of weed suppression.

## Materials and methods

The experimental site was located adjacent to the Ensis nursery at Rotorua, New Zealand (lat. 38.2°S, long. 176.3°E). In midwinter of 2004, small *B. davidii* seedlings were transplanted into single row plots (3 × 3 m) laid out in a randomized complete block design, with ten blocks and a two-row perimeter buffer. This spacing ensured that plants were not subject to competition from adjacent plants for light, water or other resources. The 40 plants within the experiment were randomly allocated to ten blocks, which included the following four treatments: (1) undefoliated control, (2) removal of 33% leaf area, (3) removal of 66% leaf area and (4) removal of 100% leaf area. For the defoliation treatments, entire leaves were removed on a monthly basis manually, to simulate the effect of insect defoliation, from late spring to late summer, initially (November) on all leaves present, and thereafter (December to February) on newly emerged leaves following the previous defoliation.

A simple process-based growth model was fitted to measurements to identify compensatory mechanisms induced by defoliation and quantify their influence on above-ground plant biomass ($W_p$) and the ratio of leaf to total biomass ($W_l/W_p$).

Above-ground biomass growth was modelled using the light use efficiency model. This model determined on a daily basis the sum of utilizable intercepted radiation from canopy characteristics (leaf area index, crown diameter), radiation and temperature. Above-ground biomass was then determined as the product

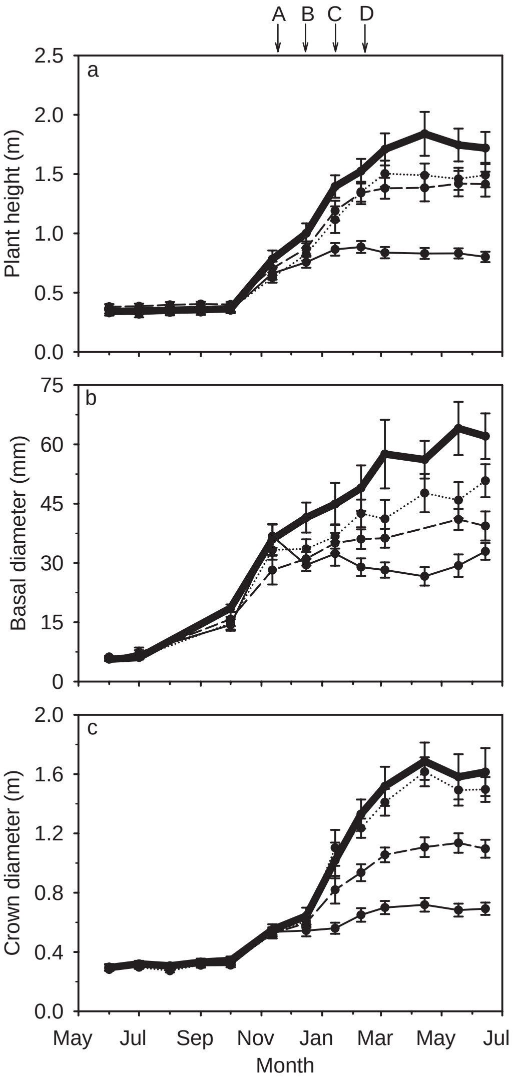


**Figure 2.** Seasonal changes in *Buddleia davidii*: (a) height, (b) basal diameter and (c) crown diameter for plants in treatments $D_0$ (thick solid line), $D_{33}$ (dotted line), $D_{66}$ (dashed line) and $D_{100}$ (thin solid line). Each point shown is the mean ± standard error of ten sample plots. The arrows A to D indicate the times of defoliations.

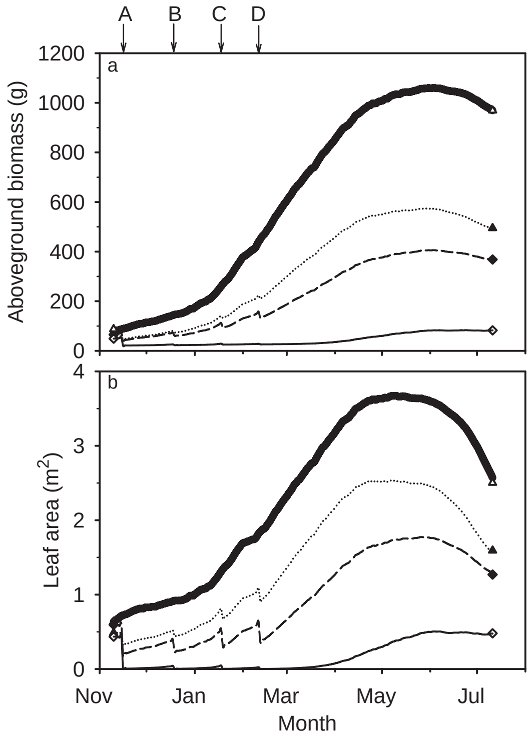


**Figure 3.** Modelled (a) above-ground biomass and (b) leaf area for $D_0$ (thick solid line), $D_{33}$ (dotted line), $D_{66}$ (dashed line) and $D_{100}$ (thin solid line). For both graphs, measured values are shown for $D_0$ (open triangles), $D_{33}$ (closed triangles), $D_{66}$ (closed diamonds) and $D_{100}$ (open diamonds). The arrows A to D indicate the times of defoliations.

of utilizable radiation and light use efficiency, and a fraction was allocated to the leaves. Both estimated leaf and biomass growth were then added to the value for the previous day to obtain cumulative total values. Estimates of plant leaf area were then determined as the product of specific leaf area and cumulative leaf mass, from which estimates of radiation interceptance and biomass growth were then made over the next time step. Full details of the derivation of the model were given by Watt *et al.* (2007).

## Results

Values of $W_p$ for treatments $D_{33,}$ $D_{66}$ and $D_{100}$ were 61%, 44% and 8%, respectively, compared with the undefoliated control ($D_0$). The defoliation treatments also resulted in significant reductions in plant height, basal diameter and crown diameter (Fig. 2). The model fitted data well (Fig. 3) and indicated that increased defoliation was also positively related to light use efficiency, daily allocation of biomass to leaves and the specific leaf area and negatively related to rates of natural leaf loss (M. Watt, unpublished data). Although the plants were able to change growth characteristics, they were unable to catch up to the control plants in the course of a single growing season.

## Discussion

*Buddleja davidii* has a strong tolerance for leaf loss, including the ability to recover from complete defoliation to a balanced allometric state in a relatively short period. This would allow it to commence growing rapidly if environmental conditions were favourable and if the cause of defoliation was removed after the initial defoliation episode. Nonetheless, there are several factors that give cause for optimism for the chances of *C. japonicus* controlling *B. davidii* under field conditions. Despite the obvious resiliency, there was a substantial reduction in plant size at the end of the experiment. If defoliation by a folivore can reduce the vigour of *B. davidii* sufficiently, then desirable vegetation may gain a competitive advantage over the weed. It is also likely that repeated defoliation over successive growth seasons would cause further depletion of energy and nutrient reserves. A separate study is examining the effect over multiple seasons.

Selection of biological control agents is very time-consuming and costly (McFadyen, 1998). The model-based approach outlined in this paper could provide a rapid cost-effective solution for assessing the likely impacts of candidate biological control agents. Once parameterized for a particular weed species from field measurements, the model could be used to examine how a large number of potential biological control agents, with a wide range of *per capita* defoliating intensities, influence growth of the target species. Given the sensitivity of net defoliation rates to agent abundance and uncertainties around the population dynamics of exotic agents before their release and establishment in a new range, it is unlikely that a precise prediction of an individual agent's success could be made using this model. However, this type of model could at least help assess the likely effects of folivores compared with agents from other guilds.

Although mechanical defoliation experiments may not accurately reflect the full range of effects of herbivores (Lehtilä and Boalt, 2004; Schooler *et al.*, 2006), they have been found to be useful for accurately assessing plant responses to various levels of defoliation (Strauss, 1988; Inouye and Tiffin, 2003; Hjältén, 2004; Raghu and Dhileepan, 2005; Wirf, 2006; Raghu *et al.*, 2006; Schooler *et al.*, 2006). Artificial and real herbivory have their respective strengths and weaknesses. Artificial herbivory can be precisely applied and does not involve any biosecurity considerations, although it may not accurately reflect the process of interest. It can also be applied in situations where the agent cannot be applied because of, say, biosecurity considerations. Conversely, real herbivory may be a more direct application of the treatment effect, but it may be difficult to achieve or measure treatment levels or covariates. Ideally, both artificial and real herbivory effects should be measured to draw on the strengths of each approach (Lehtilä and Boalt, 2004; Wirf, 2006).

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# Can a pathogen provide insurance against host shifts by a biological control organism?

**P.B. McEvoy,[1] E. Karacetin[1,2] and D.J. Bruck[3]**

*Summary*

The cinnabar moth, *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae), is an icon in population ecology and biological control that has recently lost its shine based on evidence that (a) it is less effective than alternatives (such as the ragwort flea beetle *Longitarsus jacobaeae* (Waterhouse) Coleoptera: Chrysomelidae) for controlling ragwort, *Senecio jacobaea* L. (Asteraceae), (b) it eats (harms) non-target plant species (including arrowleaf ragwort, *Senecio triangularis* Hook. (Asteraceae), a native North American wildflower, and potentially harms the animals that depend on these native plant species and (3) it carries a disease (caused by a host-specific microsporidian *Nosema tyriae*). We used a life table response experiment (LTRE) combining a factorial experiment and a matrix model to estimate the independent and interacting effects of Old World and New World host plant species (first trophic level) and the entomopathogen (third trophic level) on the life cycle and population growth of the cinnabar moth (second trophic level). Host shifts are expected if herbivore fitness is higher on novel compared with conventional host plants, perhaps because the advantage of reduced effectiveness of herbivore natural enemies outweighs the disadvantage of herbivore malnutrition associated with novel host plants. Contrary to this hypothesis, we found the population growth rate of the cinnabar moth is sharply reduced on novel compared with conventional host plants by interacting effects of disease and malnutrition. Paradoxically, a pathogen of the cinnabar moth may enhance weed biological control by providing insurance against host shifts.

***Keywords***: modelling tritrophic interactions, *Tyria jacobaeae*, pathogen–host interaction, host specificity, microspora.

## Introduction

A persistent concern hangs over the practice of classical biological control: If some biological control organisms adopt new hosts, what more can be done to contain them? A growing body of evidence suggests that phytophagous insects commonly adopt new hosts if given sufficient ecological opportunity, genetic variation in traits related to host use and fitness advantage to insects adopting new host plant species (Thompson, 2005). The cinnabar moth, *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae), introduced to control ragwort, *Senecio jacobaea* L. (Asteraceae), matches at least two of three of these requirements: ecological opportunity and genetic variation. The cinnabar moth was introduced to control ragwort on farms in lowlands of the Pacific Northwest in the United States; the unintended consequence was that it ended up feeding on native wildflowers in the mountains. The current distribution of this insect overlaps with potential non-target plant species (ecological opportunity) (Diehl and McEvoy, 1990), populations of the cinnabar moth vary in heritable traits affecting plant use (genetic variation) (Richards and Myers, 1980) and performance of cinnabar moths on one non-target species closely matches that on the target (fitness) (Diehl and McEvoy, 1990). Here we combine observational, experimental and modelling approaches to investigate how an entomopathogen might be used to contain an errant control organism. We use laboratory and modelling studies to show how an entomopathogen might be operating in this system; we use field observations on prevalence of pathogen infection in the wild to document how tritrophic interactions involving an entomopathogen species, an insect species and two plant species are operating in the field. We outline plans for future research emphasizing

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details of transmission. We conclude with implications that this research holds for the science, technology and policy of biological control.

## A model system

Biological control of ragwort has been an economic and ecological success along the west coast of North America from British Columbia to Washington, Oregon and northern California (Coombs *et al.*, 1991; McEvoy *et al.*, 1991). Ragwort has declined to 1–3% of its former abundance in that region after introduction of three insect species during a 10-year period: *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae) (cinnabar moth) starting in 1959, *Botanophila seneciella* (Meade) (Diptera: Anthomyiidae) (ragwort seed fly, formerly *Hylemia seneciella*) starting in 1966 and *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae) (ragwort flea beetle) starting in 1969. There is a potential downside as well as an upside to biological control because biological control organisms share attributes of some our worst invaders—capacity to harm, multiply, spread and evolve. The cinnabar moth is not a particularly promising biological control organism. It is less effective than alternatives (such as the ragwort flea beetle *L. jacobaeae*) for controlling ragwort (McEvoy *et al.*, 1993; McEvoy and Coombs, 1999). It eats (harms) non-target plant species (including *S. triangularis*, a native North American wildflower) (Diehl and McEvoy, 1990) and potentially harms the animals that depend on these native plant species. It carries a disease (caused by a host-specific microsporidian, *Nosema tyriae*) (Bucher and Harris, 1961; Hawkes, 1973; Canning *et al.*, 1999). We ask: can we make lemonade out of this lemon?

## Circumstances favoring host changes by the cinnabar moth

Quality of life for the cinnabar moth in the New World has declined on its Old World host plant (ragwort). First, the plant resource has collapsed. Under pressure from the ragwort flea beetle, ragwort has declined to 1–3% of its former abundance, leaving little resource for the cinnabar moth. Second, on the plant resource that remains, competitors of the cinnabar moth are overpowering it. The cinnabar moth is an inferior competitor relative to the ragwort flea beetle (McEvoy *et al.*, 1993; McEvoy and Coombs, 1999), but a superior competitor relative to the ragwort seed head fly (Crawley and Pattrasudhi, 1988). Mark–release–recapture studies show that the cinnabar moth is inferior as a colonizer on ragwort relative to both the ragwort flea beetle and ragwort seed head fly (Harrison and Thomas, 1991; Harrison *et al.*, 1995). Thus, there appears to be no possibility of coexistence of cinnabar moth with its competitors on ragwort explained by a colonization/competition trade-off (when an inferior competitor is a superior colonizer). Third, natural enemies of the cinnabar moth abound. Predators (Myers and Campbell, 1976), parasitoids (Cornell and Hawkins, 1993) and pathogens (Hawkes, 1973) have been reported to attack cinnabar moth in North America. One natural enemy, the pathogen *Nosema tyriae*, stands out as more prevalent than the rest, with a median prevalence of 70% measured across 15 populations in the states of California, Oregon and Washington in the United States (Hawkes, 1973). Diet breadth might be the cinnabar moth's ace in the hole. The fundamental host range ('physiological host range') measured in the laboratory includes 132 North American plant species and infraspecific taxa, including 20 species in Oregon (Chambers and Sundberg, 2001). Its realized host range ('ecological host range') expressed in the field appears to be much narrower. One candidate to become a new host plant, arrowleaf ragwort *S. triangularis* Hook., stands out above the rest as accessible, acceptable, suitable and vulnerable.

If the quality of life for the cinnabar moth has sharply declined on the Old World host plant species in North America, then would life be better there on a New World host plant species (taking all abiotic and biotic factors into account)?

## Tritrophic interactions

We studied interspecific interactions within a tritrophic system consisting of a host-specific pathogen, the microsporidian, *N. tyriae*; the cinnabar moth, *T. jacobaeae*; and two host plants species, the Old World host *S. jacobaea* and the New World host *S. triangularis*.

*Microspora* is a phylum of protozoa found as highly specialized, obligatory, intracellular parasites in nearly all major animal groups, being especially common in insects. They are diverse, with approximately 150 genera containing 1200 species. The disease they cause is called microsporidiosis. They possess unicellular spores, containing a uninucleate or binucleate sporoplasm and an extrusion apparatus always with a polar filament and polar cap. Transmission from one host insect to another occurs both horizontally (oral ingestion; within the same generation) and vertically (mother to progeny; between generations).

# Materials and methods

## Life table response experiment

We designed and carried out an LTRE (Caswell, 2001) to estimate the independent and interacting effects of two diets (foliage from Old World and New World hosts) and five pathogen levels (doses of 0, $10^1$, $10^2$, $10^3$ and $10^4$ spores per individual) on cinnabar moth's life cycle and population growth rate. An

LTRE combines a factorial experiment and population model as a way of linking environmental conditions, vital rates (rates of growth, development, survival, reproduction and movement) and population dynamics. An LTRE is a powerful way of translating data from individuals to implications for populations, linking a population's structure with its dynamics and analysing the demographic and population-dynamic consequences of environmental factors.

## Methods for a dose–response experiment

We collected cinnabar moth larvae for these experiments from three field sites in western Oregon, USA: Santiam Pass (44°24′08″N 121°51′01″W) in the Cascade Mountains, Basket Slough (44°57′08″N 123°16′09″W) in the Willamette Valley and Neskowin (45°6′23″N, 123°58′46″W) on the Pacific Coast, anticipating that there might be genetic variation in cinnabar moths from different geographic locations that could affect insect–plant interactions. We collected infected larvae from a single population (Neskowin, OR). Infected and uninfected larvae were reared together to facilitate horizontal transmission of the pathogen. Microsporidium spores were isolated from infected larvae and suspended in distilled water at different concentrations (0, $10^1$, $10^2$, $10^3$ and $10^4$ spores/µl). Spore suspensions were stored at 5 ± 2°C for at most 2 months. We used the same mixtures for every test unit (individual larva) regardless of the diet. *Nosema tyriae* was introduced along with the cinnabar moth, and only a single *Nosema* sp. (with unusually small spores) is known to occur in this insect. The microsporidium infecting the cinnabar moth collected from Neskowin matches the species description for *N. tyriae* (Canning *et al.*, 1999). We did not observe any insects infected with *Nosema* sp. We reared insects under optimal conditions (long day, 16:8 h L/D; temperature, °C, 25:15 L/D; humidity, 90%), reared individually (1 oz cup) and fed them *ad lib*. There were two diets (foliage of Old World and New World hosts) × five pathogen doses per individual (spore concentrations, 0, $10^1$, $10^2$, $10^3$ and $10^4$ spores) = 10 treatment combinations. We collected New World host plant (*S. triangularis*) leaves from Mary's Peak (44°30′16″N, 123°33′00″W). We grew the Old World host plant (*S. jacobaea*) in our greenhouse in individual pots—natural day lengths, temperature (°C, 25:15 L/D), humidity (90%). Leaves from both plants were fresh. We reared uninfected larvae individually through the first and second instars on both New and Old World host plants and then fed newly molted third instars 2-$mm^2$ leaf disks topically treated with 1 µl of each spore dose, corresponding to a pulse of horizontal transmission. We followed insect development daily for nearly two generations, allowing for vertical transmission. We measured vital rates of growth, development, survival and reproduction in response to diet and pathogen treatments.

## Construction and analysis of a matrix population model

We constructed and analysed a linear deterministic matrix model $N(t + 1) = A\,N(t)$, where $\boldsymbol{N}(t)$ and $\boldsymbol{N}(t + 1)$ represent vectors of the abundances in each stage from one time step ($t$) to the next ($t$ + 1) and **A** the projection matrix. The life cycle graph (Figure 1) illustrates the eight life cycle stages representing egg, five larval stages, pupa and adult. The life cycle graph also illustrates the 16 life cycle transitions in the model, with seven representing growth $g$, eight representing stasis $s$ and one representing fertility $f$. The time step in the model is 1 day. The life cycle graph can be represented as an 8 × 8 matrix **A**, which, in turn, can be used to project the dynamics.

$$A = \begin{pmatrix} s_{1,1} & 0 & 0 & 0 & 0 & 0 & 0 & f \\ g_{2,1} & s_{2,2} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & g_{3,2} & s_{3,3} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & g_{4,3} & s_{4,4} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & g_{5,4} & s_{5,5} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & g_{6,5} & s_{6,6} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & g_{7,6} & s_{7,7} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & g_{8,7} & s_{8,8} \end{pmatrix}$$

The factorial experiment yielded parameter estimates for 20 matrices, one matrix for each of ten

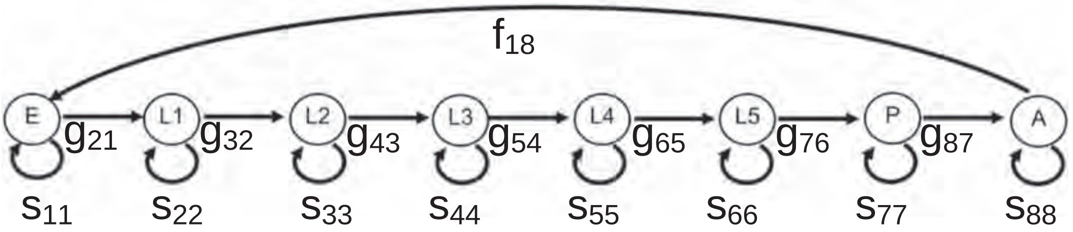


**Figure 1.** Life-cycle graph showing the eight stages and 16 transitions in the matrix model used to project cinnabar moth population growth. The eight life-cycle stages are egg (E), five larval stages (L1, L2, L3, L4 and L5), pupa (P) and adult (A). The 16 life-cycle transitions in the model include seven representing growth $g$, eight representing stasis $s$ and one representing fertility $f$. The time step in the model is 1 day.

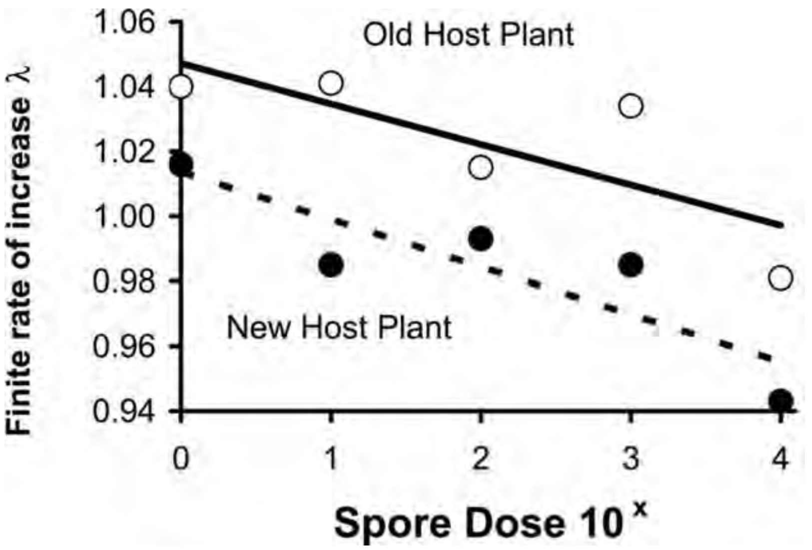


**Figure 2.** The relationship between population growth (finite rate of increase $\lambda$) of the cinnabar moth population and the treatment factors diet (foliage of New and Old Host plant species) and pathogen infection (spore dose) for the case of horizontal transmission only.

treatment combinations (two diets × five pathogen doses) × two transmission assumptions (case 1, horizontal transmission only; case 2, horizontal and vertical transmission combined). The finite rate of increase $\lambda$, the dominant eigenvalue associated with each matrix, was used as the response variable (population growth rate) in our experiment.

# Results

## Case 1: Horizontal transmission only

Population growth rates of the cinnabar moth declined with increasing *Nosema* spore dose; the negative slope of this relationship indicates that the pathogen has adverse effects (Figure 2). The New World host (*S.*

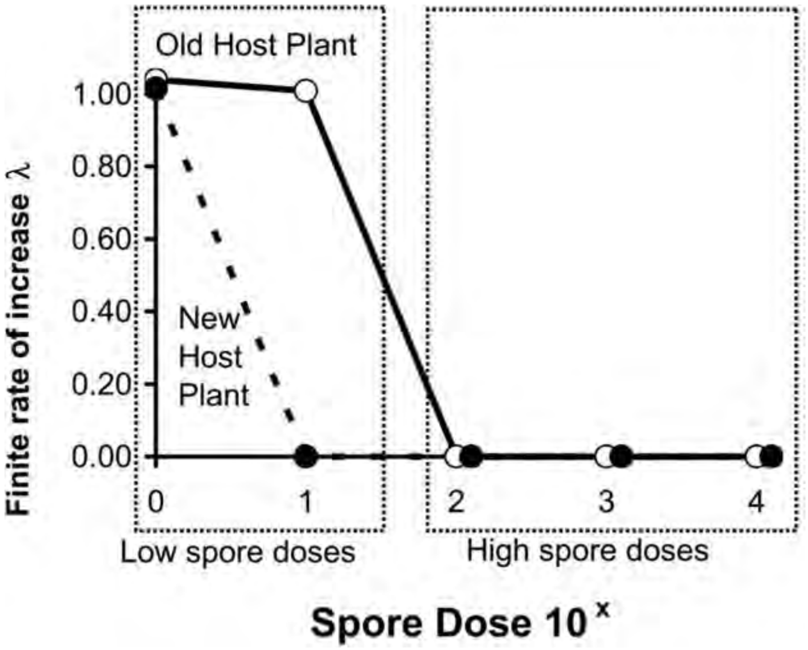


**Figure 3.** The relationship between population growth (finite rate of increase $\lambda$) of the cinnabar moth population and the treatment factors diet (foliage of New and Old Host plant species) and pathogen infection (spore dose) for the case combining horizontal and vertical transmission.

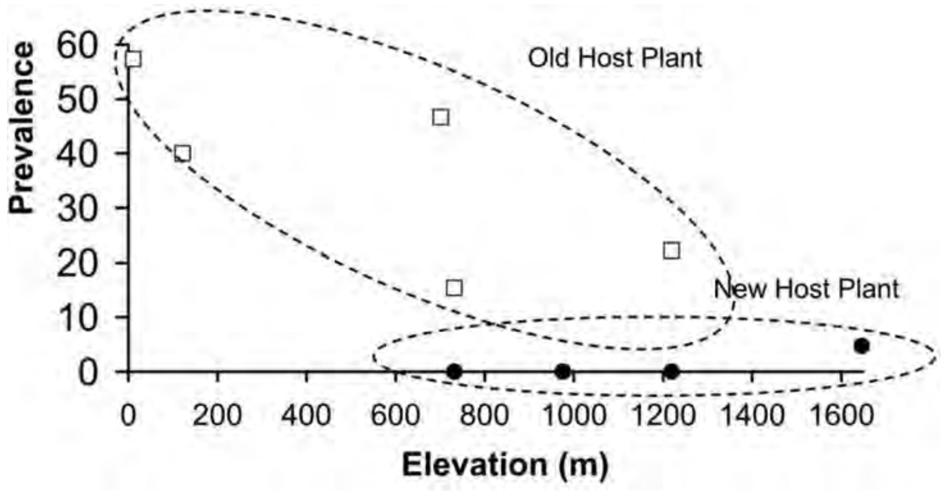


**Figure 4.** The relationship between prevalence of the pathogen *Nosema tyriae* and elevation in meters for cinnabar moth populations on Old and New Host plant species. Prevalence is measured as the percentage of host individuals infected by the pathogen within each host population.

*triangularis*) was inferior to the Old World host (*S. jacobaea*) as food; the lower intercept indicates that population growth was lower on New World as compared with Old World host species. The lines for each host are parallel, suggesting that diet and pathogen do not interact in their effects. However, qualitative description of the relationship among population growth, spore dose and host plant species changes when we increase realism by adding vertical transmission.

## Case 2: Horizontal and vertical transmission combined

When we combined horizontal and vertical transmission, diet and pathogen interacted in their effects (Figure 3). At low spore doses (left side of the graph), there was no detectable effect of pathogen infection in caterpillars on the Old World species and devastating effect of pathogen infection on the New World host species. The host plant species effect was nil in uninfected insects and huge in infected insects. At high spore doses (right side of the graph), the effect of pathogen infection was so overpowering that no effect of diet (host plant species) was expressed.

To summarize the results thus far, mild pathogen infections were devastating on New World host plants and inconsequential on Old World host plants. By contrast, severe pathogen infections were devastating on both New and Old World host plant species.

## Field Observations

Field prevalence of the pathogen varied with elevation and host plant species (Figure 4). Prevalence declined with increasing elevation (associated with decreasing temperature) over a range in elevation from 0 to 1645 m. At similar elevations, there was a higher prevalence of disease in insects on the Old World host plant species compared with the New World host plant species.

# Discussion

The strength of the pathogen–insect interaction depends on the plant species—it is weaker on the Old World host (*S. jacobaea*) than on the New World host (*S. triangularis*) for mild infections in the laboratory environment. In other words, mild infections are relatively benign in cinnabar moth populations on Old World hosts while comparatively virulent in cinnabar moth populations on New World hosts, under identical optimal laboratory conditions. This asymmetry tilts the odds against the non-target host being more acceptable or more suitable than the target, especially if cinnabar moth is given a choice between Old World and New World host plant species.

A remaining challenge is to reconcile our laboratory and field results. If a pathogen is relatively influential in the laboratory and relatively rare in the field on New World compared with Old World host plants, it would be wrong to conclude that the pathogen is not influential in insects on novel host plants in the field. Pathogens tend to die out as their hosts become rare: but are cinnabar moths rare because of past epizootics, cool temperatures, unsuitable hosts or some other causal factor(s)? Mathematical theory of pathogen–host interactions (Anderson and May, 1981) suggests that (1) there is a minimum, threshold host population size needed for persistence of a pathogen and (2) intermediate levels of virulence are optimal for increase of pathogen prevalence. It follows that higher extinction rates of the pathogen might be expected if, consistent with our observations, the pathogen is more virulent

and cinnabar moths is rarer (due to some combination of disease and malnutrition) on New World compared with Old World host plants at a given elevation (and corresponding ambient temperature).

Cause and effect cannot be established by passive observation. To investigate a feedback relationship, we need to interrupt the feedback. It would be useful to create an outbreak of cinnabar moths at high elevations and see if microsporidian epizootics develop. It would be useful to know why cinnabar moth populations are smaller at high elevations (>800 m), whether due to past epizootics, cool temperatures or unsuitable hosts. The ability of pathogens to kill ectothermic hosts has been shown to depend on host body temperature, which fluctuates with environmental conditions (Thomas and Blanford, 2003). The thermal sensitivities of plant, insect and pathogen vital rates must all be taken into account when weighing the outcome of tritrophic interactions. But for the moment at least, it seems that entomopathogens can help prevent non-target effects in the event that an insect biological control agent strays from its target host.

Finding ways to rein in errant classical biological control organisms is likely to be difficult and costly. It is better to predict and prevent adverse effects than to try to mitigate them after the fact. Some scientists worry that new organisms released into the environment are a potent form of pollution: not only with the power to have adverse effects on the environment (like chemicals), but with powers of evolution, replication and autonomous dispersal (unlike chemicals) that make adverse effects harder to predict and manage. The same scientists worry that the epidemic of plant and pest invasions is still not under control. Biological control should help in the war on weeds. Classical biological control has had the advantage over other control methods: it is a technology that operates on a scale that matches the scale of the problem. The obvious bears repeating: do not make things worse by moving the cinnabar moth and other risky control organisms to new geographic areas containing potential non-target species; that would be counterproductive.

## Acknowledgements

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# Which haystack? Climate matching to narrow the search for weed biological control agents

**M.P. Robertson,[1] C. Zachariades[2] and D.J. Kriticos[3]**

*Summary*

The shrub *Chromolaena odorata* (L.) King and Robinson (Asteraceae) is highly invasive in south-eastern Africa and is the subject of a South African biological control programme. The biotype of *C. odorata* growing in South Africa differs in several respects from the more common type noted to be invasive elsewhere, including its apparent better adaptation to a cool climate. One challenge facing the biological control programme is the identification of agents that are both suited to develop on this host biotype and persist in the relatively cool conditions found in South Africa. *C. odorata* is native to the Americas, where it has a very extensive distribution spanning a wide range of climates. Two climate matching computer programmes (CLIMEX and FloraMap) were used to focus the agent search effort by identifying areas in the Americas that are climatically similar to the invaded region in southern Africa (SA). Several higher-latitude and higher-altitude areas in South and Central America were identified by both CLIMEX and FloraMap as being similar to the region invaded by *C. odorata* in South Africa. In many areas, the two models agreed, but in others, there were discrepancies, which are discussed. There was little overlap between the region from which the SA biotype is thought to have originated and climatically suitable/similar areas in the Americas indicated by either model.

***Keywords***: agent selection, *Chromolaena odorata*, CLIMEX, FloraMap.

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# Nutritional characteristics of *Hydrilla verticillata* and its effect on two biological control agents

**J.F. Shearer, M.J. Grodowitz and J.E. Freedman**

*Summary*

A complex of abiotic and biotic factors is known to impact the establishment and success of biological control agents. Experiments using the ephydrid fly *Hydrellia pakistanae* Deonier have demonstrated that hydrilla, *Hydrilla verticillata* (L.f.) Royle, containing low protein content appears to impact larval development time and the number of eggs oviposited per female. Eggs per female were over twofold higher for larvae reared on hydrilla containing 2.4-fold more protein. Mean adult female fly weight peaked when emergence is low (i.e. low crowding) and leaf protein content is high. The hydrilla biological control pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski also responds to plant nutritional condition. The nutritional status of hydrilla shoots affects *M. terrestris* vegetative growth, disease development and conidia and microsclerotia production. High protein content in shoot tissues was associated with a more than threefold increase in conidia production and maximum disease severity. In contrast, low protein content in shoot tissues stimulated a 3.7-fold increase in melanized microsclerotia, reproductive structures that are more persistent in the environment than conidia. These studies suggest that the nutritional condition of target plants cannot be excluded as an important factor in efficacy of biological control agents. Both agents responded to favorable conditions by reproducing prolifically, which ultimately resulted in increased host damage.

***Keywords***: *Hydrellia pakistanae*, *Mycoleptodiscus terrestris*, evaluation.

## Introduction

In aquatic systems, there is scant information on the impact of biological control agents relative to the physical and nutritional characteristics of submersed aquatic macrophytes. Two agents of hydrilla, *Hydrilla verticillata* (L.f.) Royle, the Asian leaf-mining fly *Hydrellia pakistanae* Deonier (Diptera: Ephydridae) and the pathogenic fungus *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (Ascomycota: Magnaporthaceae), perform extremely well under laboratory, greenhouse and experimental conditions (Doyle *et al*., 2002; Shearer, 2002; Shearer and Nelson, 2002; Grodowitz *et al*., 2003a,b; Owens *et al*., 2006; Shearer and Jackson, 2006) but at times are inconsistent in their ability to successfully reduce hydrilla populations under field conditions. Since 1987, more than 20 million *H. pakistanae* individuals have been released with established populations occurring in Florida, Arkansas, Alabama, Georgia and Texas (Center *et al*., 1997; Julien and Griffiths, 1998; Grodowitz *et al*., 1999). Field establishment has generally been excellent with close to 90% establishment observed (Center *et al*., 1997; T. Center, unpublished data). Populations are now found far removed from their original release sites, indicating the fly is spreading naturally throughout the southeastern United States. Significant *Hydrellia* spp. impact has been observed at sites in Texas, Florida and Georgia (Grodowitz *et al*., 2003a,b) but significant increases in fly populations and subsequent impact have not occurred at many sites. Reasons are not completely understood.

*Mycoleptodiscus terrestris* reproduces asexually by thin-walled conidia and by melanized survival structures called microsclerotia. To date, sexual reproduction of the fungus has not been observed, therefore sexual spores were not an issue in this study. Conidia develop from spore-producing structures called sporodochia following ingress by the pathogen. The sporodochia form on tissue surfaces within 5 to 7 days postinoculation

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followed within a day by commencement of spore production. It has been documented that spore production may vary in relation to the substrata available and to environmental variables such as stress or disturbance (Dix and Webster, 1995). Under optimum conditions in greenhouse studies, the hydrilla pathogen *M. terrestris* is consistently pathogenic to hydrilla and can reduce shoot biomass by 97% to 99% (Shearer, 2002). However, subjecting field populations of hydrilla to similar rates of *M. terrestris* inoculum has often produced inconsistent results.

Potential factors that might limit agent performance on hydrilla include parasites, predators, temperature, water flow, turbidity, plant density, age and plant nutritional status. To better understand the importance of plant nutrition on agent performance, hydrilla plants of

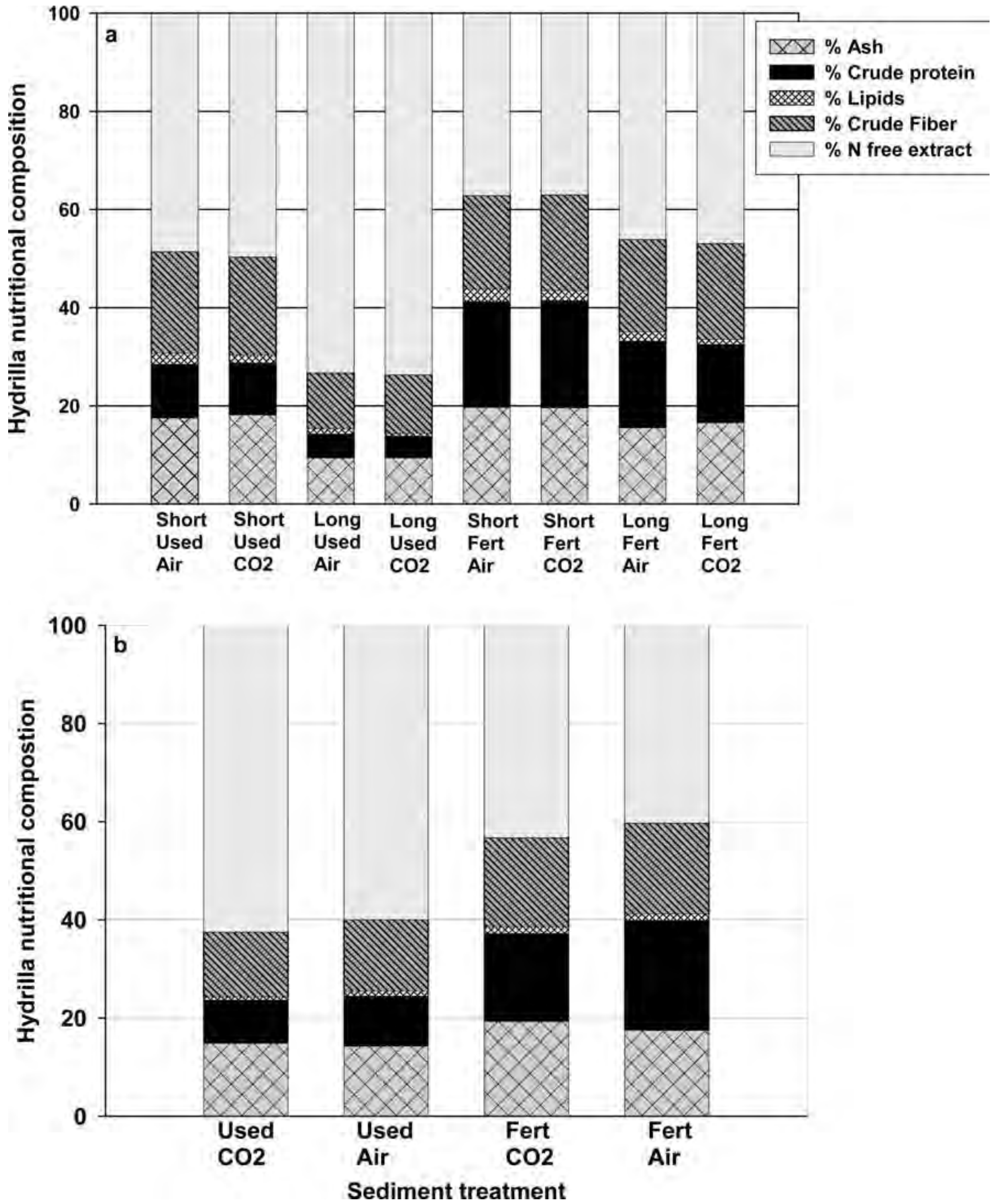


**Figure 1.** Proximate analysis of hydrilla shoot tissues for (a) insect and (b) pathogen study. Sediments were nutrient-deficient (Used) or nutrient-enriched (Fert = fertilized). Plants received ambient air (Air) or air enriched with carbon dioxide ($CO_2$). Growth periods were 4 weeks (Short) or 10 weeks (Long).

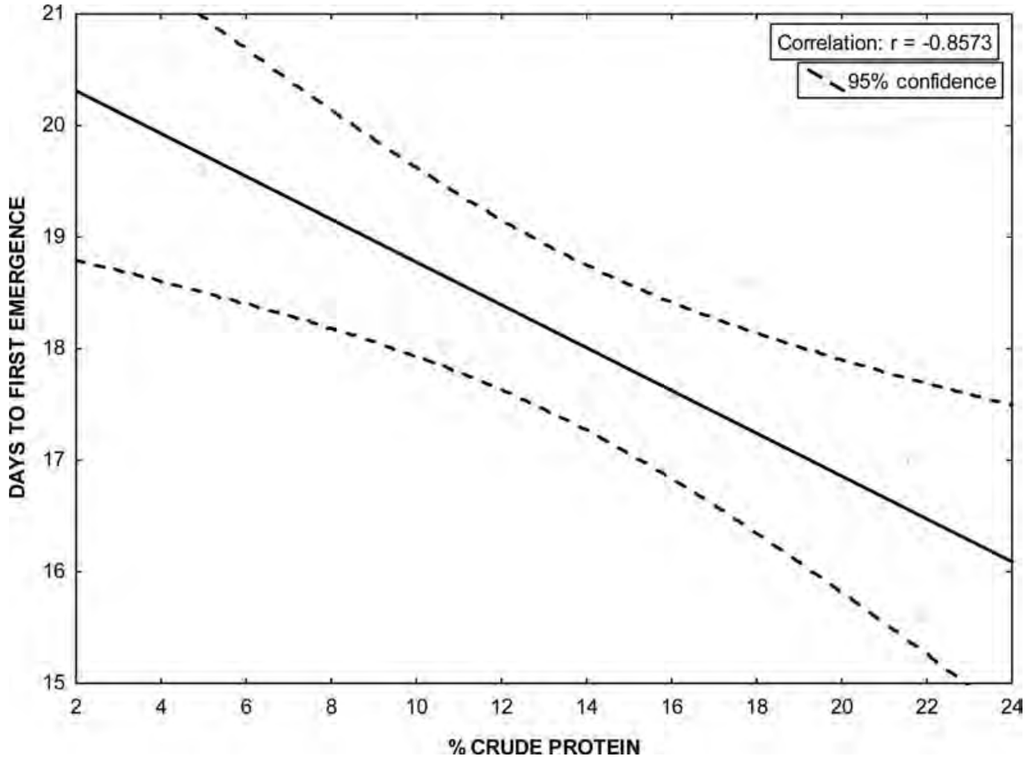


**Figure 2.** Correlation between crude protein and days to first emergence of *Hydrellia pakistanae*.

varying nutritional status were challenged with *H. pakistanae* and *M. terrestris*.

## Materials and methods

### Plant growth

Hydrilla plants of known nutritional composition were produced by growing them in used or fertilized sediments under different aeration conditions (high or low $CO_2$) using procedures described by Grodowitz and McFarland (2002) and Shearer *et al.* (2007). The used sediment was rendered nitrogen-poor because of previous growth of submersed macrophytes. Fertilized sediments were amended with 0.7 g $NH_4Cl$ per liter of wet sediment. Additionally, for the insect experiment, period of growth was varied (long vs. short) to produce plants having varying degrees of leaf hardness as measured by a penetrometer. Nutritional parameters, including percent ash, crude protein, ether-extractable compounds, crude fiber and nitrogen-free extract, were determined using a standard feed analysis known as a proximate analysis described in detail by Grodowitz and McFarland (2002). Phosphorous concentration was determined using atomic absorption techniques.

### Insect biological control agent

Insects were reared in a greenhouse, beginning with 50 eggs per container, on hydrilla plants of varying nutritional composition in 3.5-l containers in a water bath maintained at 22–25°C (Freedman *et al.*, 2001). Emerged adults were removed from the containers daily and released into oviposition chambers (30.5 × 30.5 × 30.5 cm). Percent emergence was calculated. Each treatment was replicated five times.

Within the oviposition chambers, females were allowed to oviposit freely onto five to seven hydrilla apical shoots held within an open 100 × 15-mm (d × h) Petri dish containing deionized water. After the adults died, the sex ratio was recorded and dessicated females were weighed. Hydrilla shoots were removed from the oviposition chambers every 3 to 5 days, eggs were identified and counted and number of eggs per female was calculated.

### Pathogen biological control agent

Hydrilla apical shoots (5 cm) of variable nutritional compositions were placed in 250-ml Erlenmeyer flasks containing 150 ml sterile water and 20 µl wet inoculum. Inoculum was prepared as described by Shearer *et al.* (2007). Control flasks received an additional 20 µl of sterile water. Each treatment was replicated five times. The flasks were randomly arranged on a rotary shaker (Innova 2300, New Brunswick Scientific, Edison, NJ) set at 50 rpm and incubated at room temperature for 2 weeks.

At 7 and 14 days postinoculation, the hydrilla shoots were visually assessed for disease development based

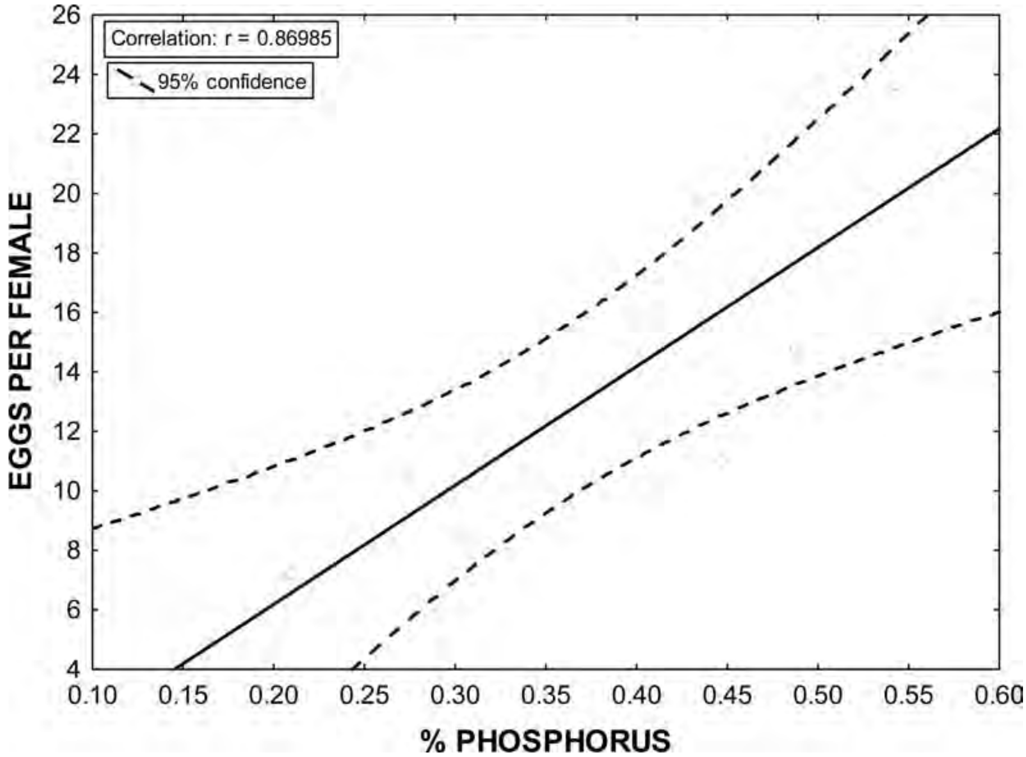


**Figure 3.** Correlation between hydrilla phosphorous content and eggs per female for *Hydrellia pakistanae*.

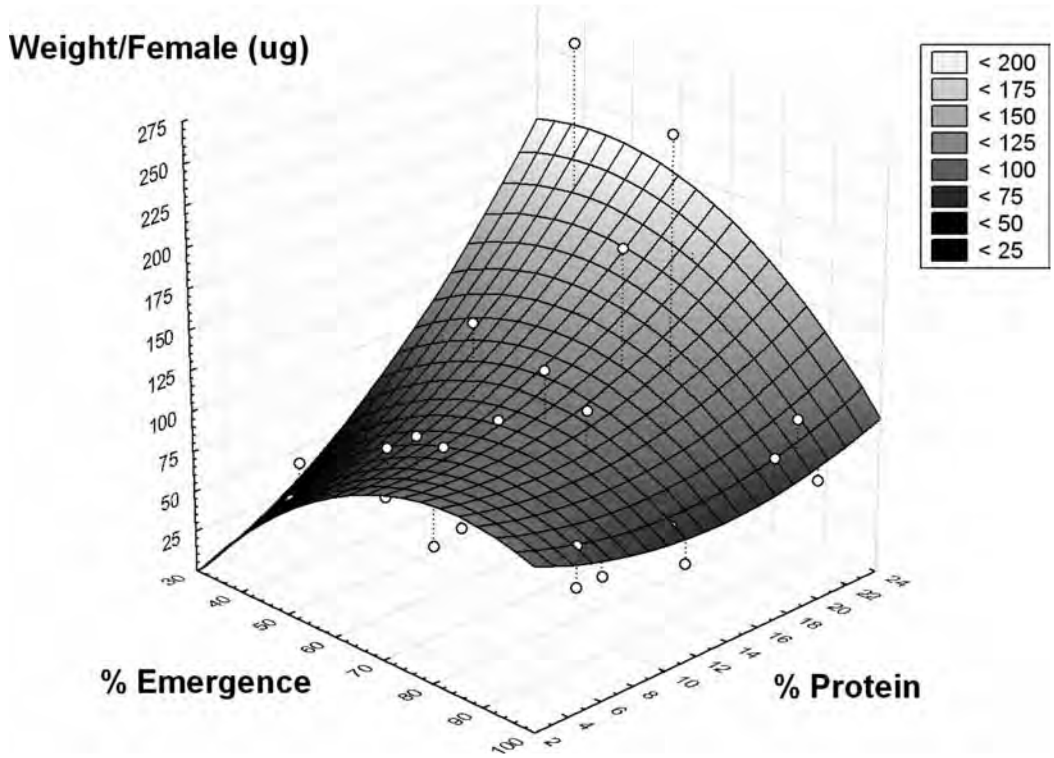


**Figure 4.** 3-D surface plot with data points marked for percent emergence of *Hydrellia pakistanae* vs weight per female and leaf protein content.

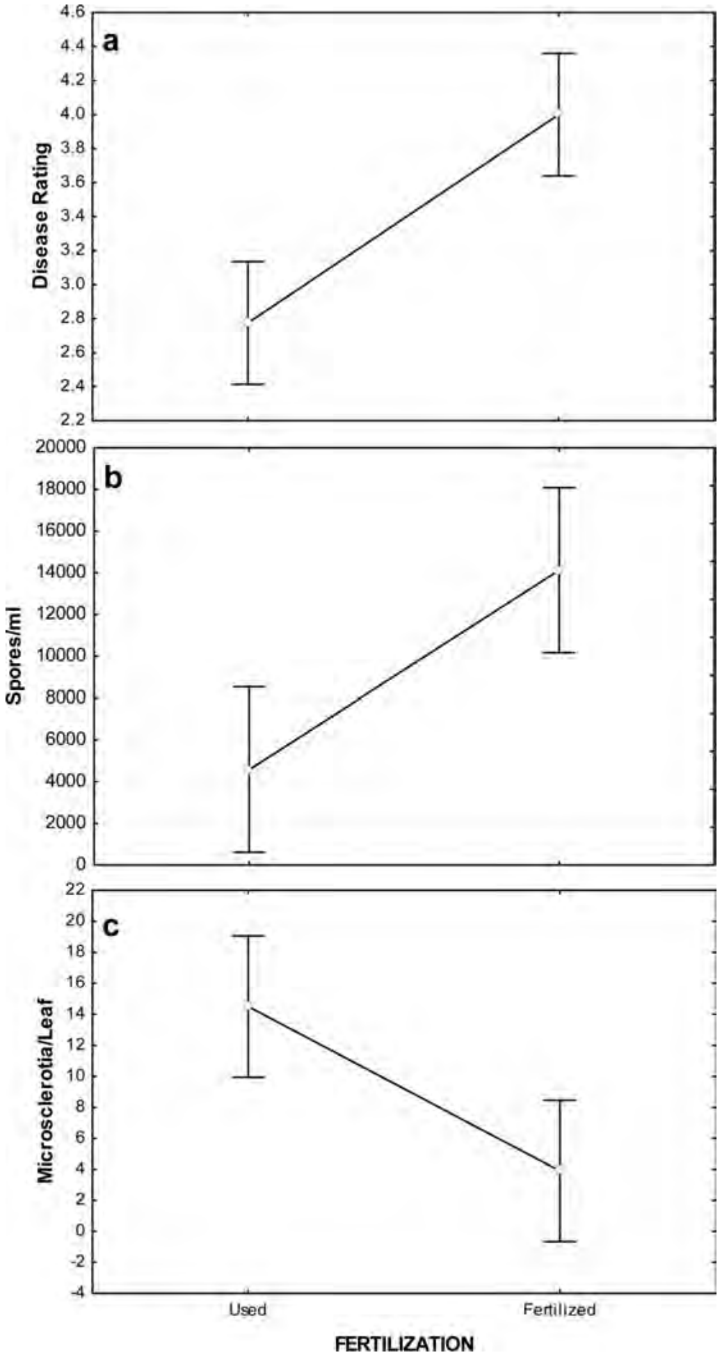


**Figure 5.** Effects of hydrilla fertilization levels on *Mycoleptodiscus terrestris* (a) disease development, (b) asexual spore production in the form of conidia and (c) production of survival structures or microsclerotia.

on a disease rating scale from 0 to 4, where 0 = green and healthy, 1 = slight chlorosis, 2 = general chlorosis, 3 = tissues flaccid and disarticulating and 4 = complete tissue collapse. At 14 days postinoculation, the flasks were gently shaken to dislodge any spores that had developed on infected tissue surfaces. The number of spores released into the water was then determined using a hemacytometer. Three leaves were randomly retrieved from each flask to count microsclerotia that had developed within leaf tissues.

## Statistical analysis

Statistical analyses were performed using Statistica version 7.1 (Statsoft, 2005) and included ANOVA, correlation analysis and a distance-weighted least square means graphing technique to visualize three-dimensional trends with corresponding measures of the amount of variance explained (i.e. $R$). Statistical significance was assumed at or below $P = 0.05$, unless otherwise noted.

# Results and discussion

## Plant nutritional status

By manipulating growing conditions, hydrilla plants were produced with significant differences in nutritional composition for percent nitrogen-free extract (soluble sugar, starch and some hemicelluloses), crude fiber (cellulose and some lignin), ether-extractable compounds (lipids and fats), crude protein (total nitrogen) and ash (mineral content) (Fig. 1). Of particular note was that crude protein, as a measure of total nitrogen, was approximately twofold higher in plants grown in fertilized sediments compared with plants cultured in used or nutrient-depleted sediments. Protein levels were similar for corresponding treatments for plants used for both the insect and pathogen experiments.

## Insect response to plant nutrition

Significant difference in days to first emergence (an indication of development time) was noted for both the fertilized ($df = 1, 32$, $P = 0.0009$) and growth period ($df = 1, 32$, $P = 0.001$) main effects only. Time to first emergence was 2 days shorter in fertilized sediments as compared with used sediments and 2 days longer for plants grown for longer periods under cooler temperatures compared with shorter growth periods at higher temperatures. As expected, a significant correlation between percent crude protein and days to first emergence was observed where higher crude protein values were associated with fewer days to first emergence (Fig. 2). This was not surprising, as similar results were noted in experiments conducted by Wheeler and Center (1996), where larvae reared on harder hydrilla leaves (and lower protein) resulted in longer developmental times.

Plants grown in fertilized sediments gave rise to female flies that laid more than twice as many eggs ($df = 1, 32$, $P < 0.00017$), as female flies that were reared on plants in used sediments. Mean number of eggs per female was 7.8 for used sediments compared with 17.2 for fertilized sediments. Although there was a strong linear relationship between phosphorous and protein content in plant tissues, egg production appeared to be more strongly correlated with phosphorous than protein. The $r$ values were higher when egg numbers were correlated with phosphorous (Fig. 3, $r = 0.87$) than with crude protein ($r = 0.65$).

There is an interesting relationship among weight per female (an indication of fecundity), crude protein and percent emergence as an indicator of crowding (Fig. 4). Female fly weight peaks when emergence is low (i.e. low crowding) and protein is high. However, as percent emergence increases, leading to increased crowding and competition amongst larvae, the emerging female weight remains low even at high protein levels. Hence, crowding strongly influences female weight and most likely fecundity.

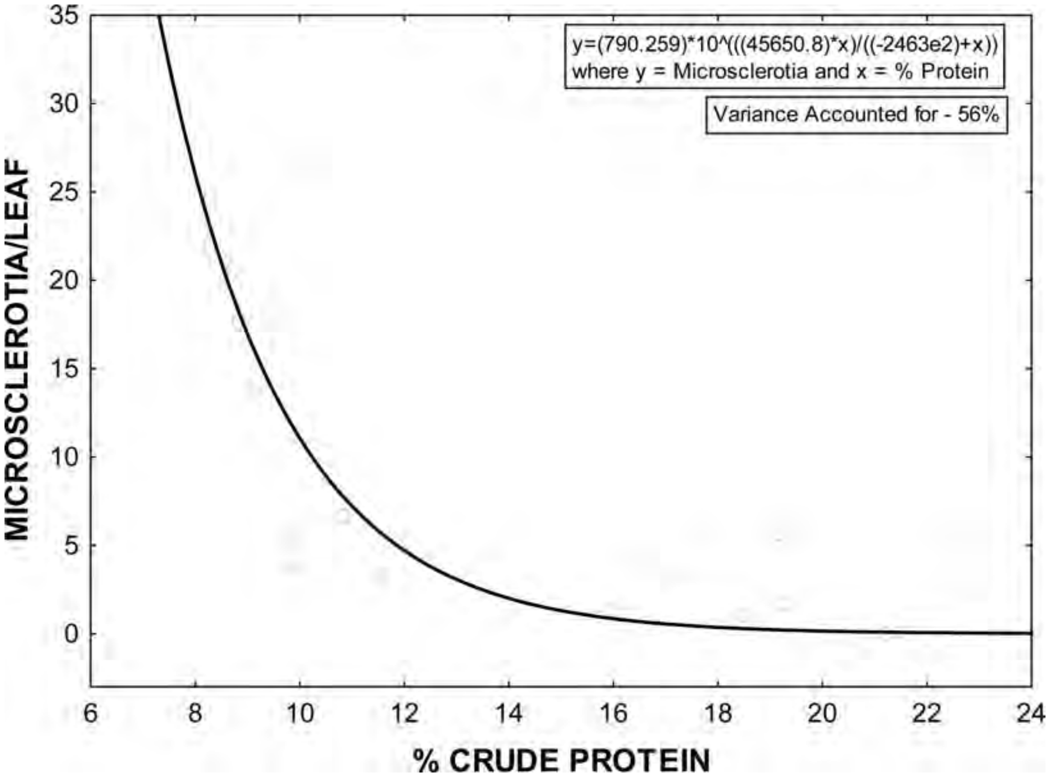


**Figure 6.** Relationship between percent crude protein in hydrilla leaf tissues and production of *Mycoleptodiscus terrestris* microsclerotia.

## Pathogen response to plant nutrition

Fourteen days postinoculation with *M. terrestris*, disease ratings between plants grown in fertilized and used sediments were significantly higher (*df* = 1, 16, *P* = 0.0001; Fig. 5a) than for plants grown in used sediments. Although the leaves of plants grown in low-fertility sediment were chlorotic and becoming flaccid, the stems remained intact. In field situations, such plants would probably recover and regrow from undamaged root crowns (Netherland and Shearer, 1996). The highest disease severity rating (Fig. 5a) was consistently found on inoculated hydrilla that had high leaf-protein content. These plants collapsed to the bottom of the flasks and, lacking cell integrity, would have had no possibility of recovery. Other studies have documented that high leaf-protein content is often associated with increases in disease severity (Ghorbani *et al.*, 2002; Latty *et al.*, 2002).

Plant nutritional status also affected the pathogen's reproductive ability. *Mycoleptodiscus terrestris* conidial production appeared to be influenced by the substrate. This is indicated by significantly higher numbers of spores produced in flasks containing hydrilla plants grown in high-fertility sediments (*df* = 1, 16, *P* = 0.0021) (Fig. 5b). In contrast to conidia, significantly higher numbers of vegetative reproductive structures, microsclerotia, were present in leaves of hydrilla plants grown in low-fertility or used sediment at 14 days postinoculation (*df* = 1, 16, *P* = 0.0028) (Fig. 5c). Lacking nutrients for continued mycelial growth, *M. terrestris*, in all likelihood, used the available nutrients in plant tissues and mycelium for production of survival structures.

The highest number of microsclerotia developed in leaves from plants that had the lowest available nitrogen. The response was strongly curvilinear, suggesting that microsclerotia production may be triggered by some threshold level of leaf protein, perhaps <9% (Fig. 6). Limited nitrogen availability apparently induced changes in the pathogen that altered growth from active proliferation, i.e. conidial production, to preparation for a period of dormancy or lack of resources, i.e. microsclerotia production. A similar curvilinear response for microsclerotia numbers and spore production was observed, indicating that such a shift had occurred (Fig. 7).

## Implications for biological control

Hydrilla plant nutrition affected the two very different biological control agents in similar ways. High hydrilla leaf-protein content stimulated agent reproduction as indicated by increased *H. pakistanae* female weight and fecundity and increased conidial production and disease severity in the case of *M. terrestris*. Low leaf-protein content in hydrilla negatively affected reproduction of both biological control agents, resulting in increased developmental times and reduced fecundity for the insect and a shift by the pathogen to a higher

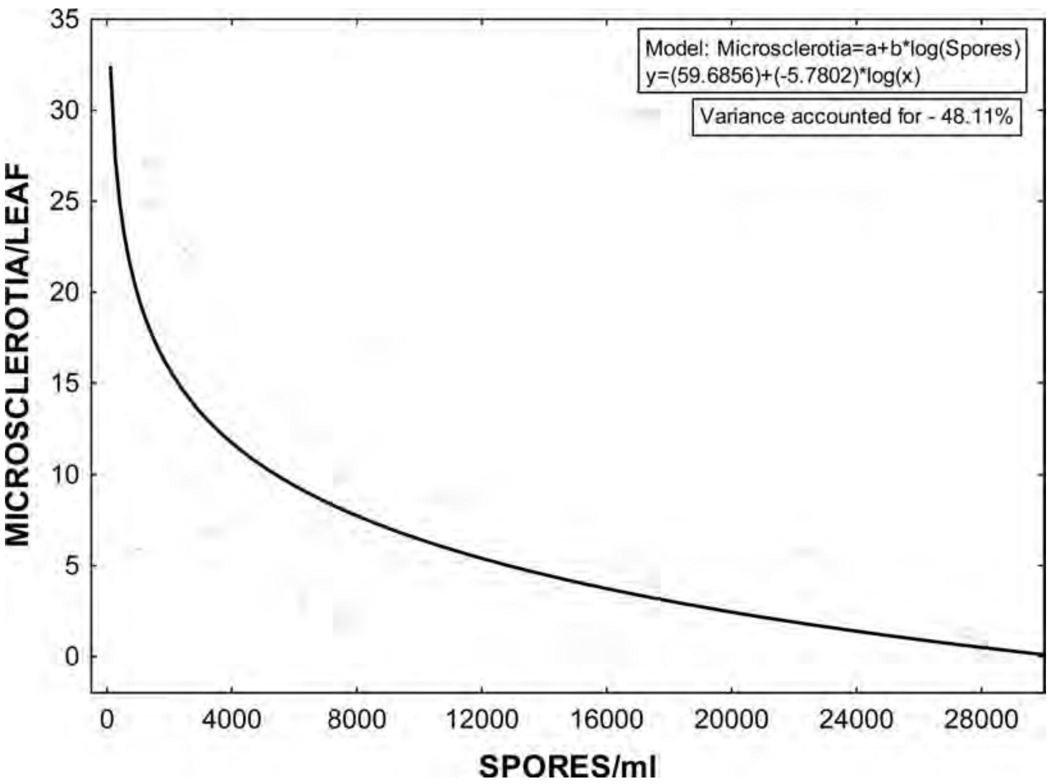


**Figure 7.** Relationship between *Mycoleptodiscus terrestris* microsclerotia and spore production.

production of microsclerotia. Based on the case studies, it appears that plant nutritional quality could have been a factor in inconsistent field results in the past and that higher quality plants should lead to increased agent establishment and impact. Support for this conclusion can be found in a study using the salvinia weevil, *Cyrtobagous salviniae* Calder and Sands, where fertilization of giant salvinia plants, *Salvinia molesta* D.S. Mitchell, in the field substantially aided the establishment of the agent and ultimately lead to a successful biological control effort (Room and Thomas, 1985).

## Acknowledgements

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# How sensitive is weed invasion to seed predation?

**R.D. van Klinken,[1] R. Colasanti[1] and Y.M. Buckley[2,3]**

*Summary*

Seed predators are typically easy to test, rear, release and establish and can be particularly useful when targeting invaders that also provide benefits to some parts of the community. However, seed predation rates are generally considered too low to cause significant population regulation of invasive plants. They may, however, impact on invasion rates (here defined as the combined effects of spread and infill), although this has proved difficult to demonstrate. In this paper, we use a cellular automaton model to test whether the effect of seed predation on the regulation of existing populations is influenced by the seed dispersal mechanism and how the addition of a seed predator to an existing population affects invasion rates. We found that population regulation occurs at significantly lower seed predation rates for poor dispersers as compared with good dispersers (existing models commonly assume random dispersal) and that seed predation impacts on invasion rate at predation rates that are commonly observed in the field. Further analysis is required to test how robust these conclusions are for different plant parameters, for a range of dispersal mechanisms (including long-distance dispersal) and in heterogeneous landscapes.

***Keywords***: agent selection, population regulation, seed dispersal mechanisms, spread rates.

## Introduction

Seed predators have a mixed record in weed biological control. They are typically easy to test, rear, release and establish and can be particularly useful when targeting beneficial plants. However, population modelling and field observations suggest that seed predation rates need to be very high to regulate plant populations, typically in the order of 90–99% seed mortality (Myers and Risling, 2000; Sheppard *et al.*, 2002; Buckley *et al.*, 2005), as invasive plants are normally microsite-limited (Crawley, 1992). Some biological control agents do cause sufficient seed mortality to regulate populations (Louda and Potvin, 1995; Hoffmann and Moran, 1998), but most biological control agents probably do not (Crawley, 1992; R.D. van Klinken, unpublished data).

Seed predators may, however, impact on invasive plant populations in ways other than population regulation per se (van Klinken *et al.*, 2004). Of those, reduced invasion rate (defined here as the combined effects of spread and infill) is likely to offer the most substantial benefits for management. Invading populations may be seed-limited rather than microsite-limited along their invading front and may therefore be most sensitive to reductions in viable seed through seed predation or other mechanisms. If biological control agents can or do reduce invasion rates, then it is important to demonstrate such benefits. However, it is difficult to do so empirically, and to our knowledge, this has not been done. However, modelling provides opportunities for testing the likely sensitivity of invasion rates to seed predation under a range of circumstances.

A range of mathematical modelling approaches have been used to test the relationship between fecundity and spread speed (Buckley *et al.*, 2005; Hastings *et al.*, 2005). However, modelling approaches used so far are not well-suited to exploring the spatial effects of demographic change and landscape heterogeneity on plant invasion (With, 2002). We therefore developed a cellular automaton model to explore the interactions amongst dispersal mechanism, seed predation and invasion. We present early results of this work, which tests

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(1) whether seed dispersal mechanisms influence the way populations are regulated by seed predators and (2) what effect adding a seed predator to an existing population has on invasion rates.

We restrict our discussion to seed predators. However, results and conclusions are equally applicable to factors that might limit seed production, including a wide range of biological control agents that attack immature reproductive organs or stress plants.

# Methods

## Model design

The model is based on a cellular automaton model of plant populations (Colasanti *et al.*, 2007). Cellular automaton models are spatial models that treat time and space in discrete elements. In our model, an iteration, which represents a single growing year, comprises plant establishment and survival, seed production and seed dispersal. Each cell of the cellular automaton can contain a single plant, which has an individual age and can produce a number of individual seeds. At maturity, a plant can produce seed but is subject to a death rate that is proportional to its age, with a probability of death of 95% after 20 years. Plant parameters were set to model a simplified perennial woody shrub (Table 1).

Seed dispersal between grid cells was modelled using a random-walk algorithm, which results in a normally distributed dispersal kernel. A proportion of seeds within each cell 'fall' to the ground, and the remainder are randomly distributed between the cell and its immediate neighbours. This algorithm is applied to each cell

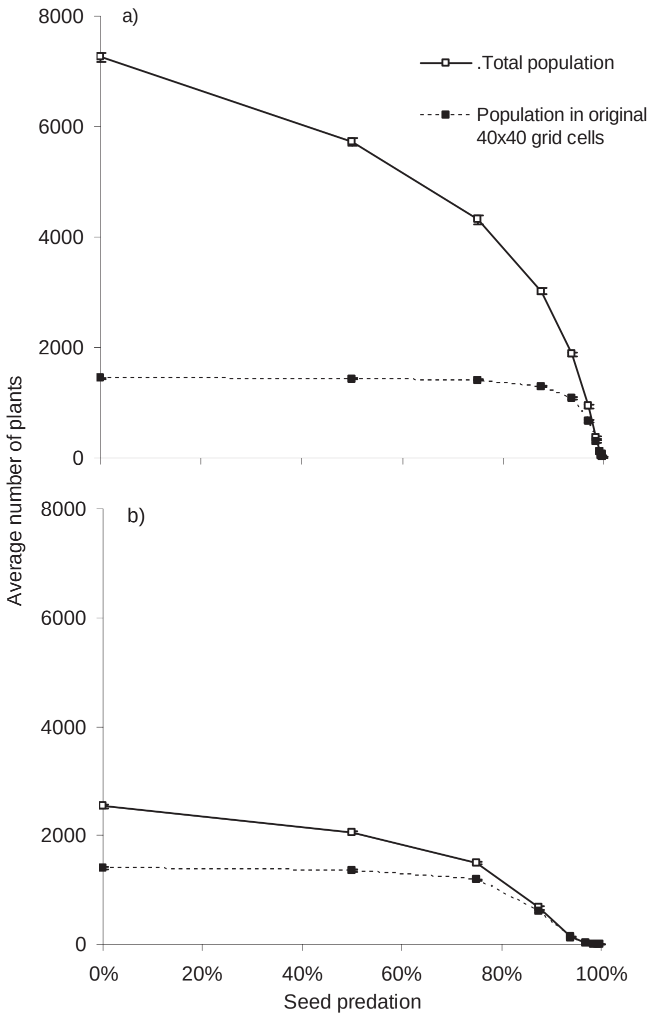


**Figure 1.** A comparison of the effect of adding seed predators to an existing 40 × 40-grid cell population (maximum population size = 1600 adults), assuming (a) good dispersal (after 25 years) and (b) poor dispersal (after 50 years) (average ± SE). Data shows the number of adults in the source area and the total adult population after 25 and 50 years of seed predation, respectively.

**Table 1.** Variables used in the model and parameter values.

| Variables | Parameters |
|---|---|
| Time step | Annual |
| Seed production | 1024/plant/year |
| Seed bank | None |
| Time to maturity | 5 years |
| Adult lifespan | 20 years (95% probability) |
| Probability to maturity (from seed) | 0.01 |
| Seed predation | 0–99% |

and repeated until there are no more seeds to distribute. The fall rate was set to 10% to model a good disperser and 90% for a poor disperser.

The effect of seed predation was modelled for an established population that occupied a 40 × 40-cell grid block in the centre of a 160 × 160-cell matrix. The number of plants that occurred within the established block was counted separately from those plants that were outside this block. This was done at the end of a fixed number of iterations (years), 25 for the good disperser and 50 for the poor disperser; the former had a much greater rate of spread and was thus stopped sooner. Predispersal seed predation was simulated by reducing the number of seeds produced by the plant from a maximum of 1024 to a minimum of 2 seeds using an exponential series (1024, 512, 256…2). The experiments for each level of seed predation (0–99%) were repeated 20 times, and the average values and standard deviations were calculated.

# Results and discussion

## Effect of seed predation on population regulation

When seed dispersal approached random, seed predation needed to be at least 90% before it began having a significant impact on plant density within the established 40 × 40-grid cell population (Fig. 1a). This threshold is sensitive to model parameters, including seed production per plant per year (here set at 1024 seeds). However, a threshold of 90% or greater is a common conclusion for population modelling.

Our model showed that seed dispersal mechanisms can affect the way in which even relatively small populations are regulated. Where dispersal was poor, less seed predation is required to have an impact on plant population density (Fig. 1b). Poor seed dispersal results in seeds being aggregated under and adjacent to parent trees, thereby producing a more heterogeneous response to seed predation across the site.

## Effect of seed predation on invasion rate

Invasion rate in our model was restricted by the surface area of the invasion front, as there were no long-distance dispersal events. It was also delayed because plants took 5 years to reach maturity. As would be expected, invasion rate was much faster for a good

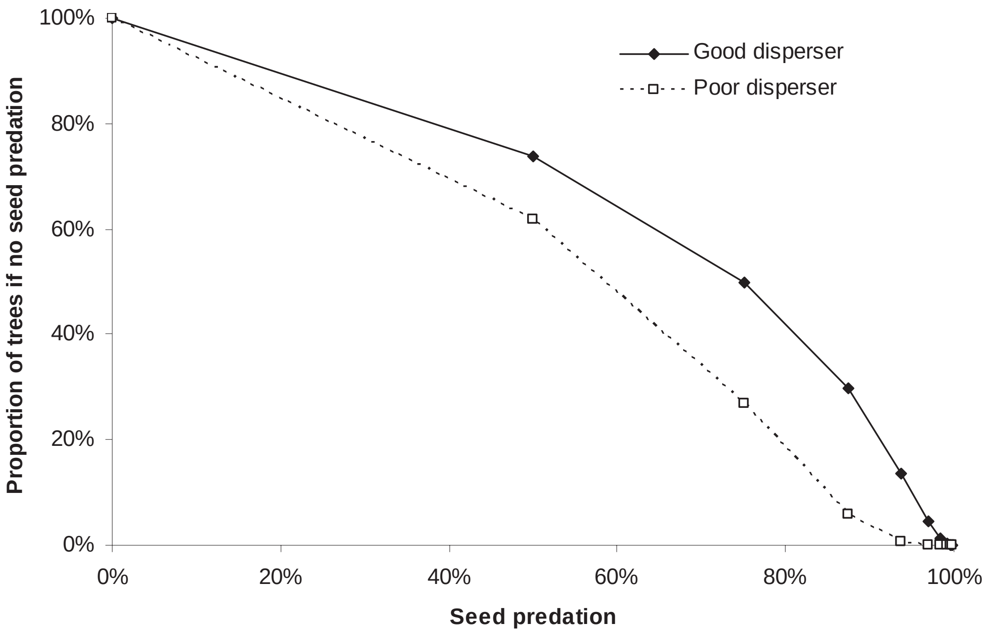


**Figure 2.** The effect of seed predation on invasion rate (as a proportion of the total number of trees present outside the source population in the absence of seed predation) assuming good and poor dispersal.

disperser (Fig. 1a), with the adult population increasing fivefold after only 25 years compared with only a 1.8-fold increase after 50 years for a poor disperser (Fig. 1b), in the absence of seed predation.

Seed predation had an immediate effect on invasion rate (as a proportion of invading trees in the absence of seed predation), with substantial reductions occurring at seed predation rates well below that required to affect population regulation (Fig. 1). The effect was, however, proportionally greatest when dispersal was poor (Fig. 2).

### Other factors that might be important

Our model is currently very simplistic and begs the question of how conclusions will differ with different plant parameters, dispersal mechanisms and landscape structure. Other models have already partially explored the effect of demographic parameters on invasion rates and found the system to be relative insensitive to realistic variation in some plant parameters, including adult longevity and time to reproduction (Buckley *et al.*, 2005). Our results show that dispersal mechanisms can clearly interact with the way seed predation affects both population regulation and invasion rates. The next step will be to test the effect of rare to frequent long-distance dispersal events. Landscape heterogeneity is also likely to be important, through its effect on recruitment probabilities (Buckley *et al.*, 2005), seed production (Paynter *et al.*, 1996) and dispersal probabilities. Other likely moderating factors include Allee effects (which result in seed production increasing with adult density), density dependence of seed predators and seed predators attacking seeds before and/or after seed dispersal.

### Implications for biological control

Our results suggest that the seed predation rates required to regulate plant populations may not need to be as high as previously thought but that it will vary with seed dispersal mechanism. However, the required seed predation rates may still be too high for most species of seed predators to realistically achieve (R.D. van Klinken, unpublished data).

In contrast, invasion rates are much more sensitive to seed reductions. Sensitivities will depend on dispersal mechanisms and landscape heterogeneity. However, our results suggest that even commonly observed seed predation rates may be sufficient to result in significantly reduced invasion rates.

The likelihood that even relatively low seed predation rates can reduce invasion rates supports the suggested practice of releasing seed predators as early as possible in the invasion process, including at the time of introduction of new agroforestry species (Zimmermann and Neser, 1999). However, further work is still required to simulate the effect of seed predation for specific species and landscapes.

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# Altered nutrient cycling as a novel non-target effect of weed biocontrol

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Invasive weeds have been shown to alter decomposition rates and nutrient cycling in invaded systems. However, little attention has been paid to the role of introduced biological control agents in influencing nutrient cycling. Alligator weed (*Alternanthera philoxeroides*) invades waterways in northern New Zealand. It is partially controlled in these environments by the alligator weed flea beetle, *Agasicles hygrophila*, which was introduced as a biological control agent in the early 1980s and has since become widespread. Annual biomass dynamics and litter decomposition rates of alligator weed were compared with those of two native sedges, *Schoenoplectus tabernaemontani* and *Isolepis prolifer*, in a New Zealand lake. Herbivory by the alligator weed flea beetle caused a 75% decrease in alligator weed above-ground biomass over a 3-month period in late spring and early summer. This contrasted in both timing and magnitude of litter input by either native plant species. In addition, alligator weed litter decomposed significantly faster than the litter of either native species. This combination is likely to alter the annual availability of nutrients within this ecosystem, with potential flow on effects including facilitation of further weed invasion.

# Interactions of plant quality and predation affect the success of purple loosestrife biocontrol programme

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Biocontrol of purple loosestrife (*Lythrum salicaria*) in North America is generally considered highly successful. Yet, at certain sites, biocontrol agents fail to control their host plant. Field observations indicate that leaf-feeding biocontrol agents *Galerucella pusilla* and *Galerucella calmariensis* are more abundant in flooded than in dry sites. We tested two hypotheses: (1) leaf beetles suffer increased predation in dry areas and (2) superior plant quality in flooded areas results in improved leaf beetle performance. To test Hypothesis 1, we conducted predator exclusion experiments at multiple sites. We found marginal effects of exposure to predation on leaf beetle survival, but survival was always higher under flooded conditions. To test Hypothesis 2, we conducted a common garden experiment where we grew *L. salicaria* plants under three water levels. We measured several plant traits that are potentially related to plant quality and leaf beetle performance. Plants grown in flooded treatments had higher water content and lower tannic acid concentration. All other traits were not significant. Consistent with field observations, leaf beetle fertility and survival were higher on flooded plants. Our data suggest that the relative effects of top–down (predation) vs. bottom–up forces (plant quality) vary along a water level gradient and may further interact with abiotic conditions.

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# An arthropod and a pathogen in combination as biocontrol agents: how do they shape up?

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The South African biological control programme of Crofton weed (*Ageratina adenophora*) was initiated in 1984 with the release of a stem galling fly, *Procecidochares utilis* (Diptera: Tephritidae), and a leaf spot pathogen, *Phaeoramularia eupatorii-odorati* (1987) (Hyphomycetes: Moniliales). A postrelease evaluation at three geographically different sites shows that the pathogen is widespread throughout the country, with up to 95% of plants being infected. However, the severity of infection is relatively low, with less than 50% of leaves on individual stems infected. The fly is not widespread in *A. adenophora* infestations. Where it occurs, 30% of the stems are galled and only 10% exhibit repeated galling. Parasitism and phenological asynchrony with the plant are shown to suppress fly numbers. Neither the fly nor pathogen, individually or in combination, significantly affects vegetative growth of the weed. However, low seedling densities at sites with the fly suggest it may reduce the reproductive potential of *A. adenophora*. Mechanical removal is shown to reduce the density of adult plants but promote recruitment of seedlings and is costly. Therefore, further agent selection is recommended for the control of *A. adenophora* in South Africa.

# Impact of invasive exotic knotweeds (*Fallopia* spp.) on invertebrate communities

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Exotic knotweeds (*Fallopia* spp.) are considered to be amongst the most serious invasive exotic weeds in Europe, causing significant damage to native ecosystems. However, with the exception of competitive exclusion of native vegetation, their suggested ecological impact is poorly supported by experimental studies. We investigated the ecological impact of exotic knotweed species in selected areas of France, Germany and Switzerland. Ten locations were selected along river courses with different levels of knotweed infestations and invertebrate traps (pitfall and combi traps) were randomly established in vegetation invaded by exotic knotweed, as well as in vegetation that can potentially be invaded by knotweed (open and bush-dominated vegetation). Results indicate that invasion by exotic knotweeds does not only have strong effects on native vegetation but also affects invertebrate communities. The overall abundance, biomass and diversity of invertebrates in plots invaded by exotic knotweed were strongly reduced compared with control plots. Exotic plant species are in general introduced without their specific natural enemies and are also often less palatable to generalist herbivores. In accordance with this, we found reduced diversity for herbivore invertebrates in knotweed patches. In addition, a negative effect of exotic knotweeds could also be detected in other trophic groups (e.g. predators, detritivores).

# An experimental test of the importance of climate matching for biological control introductions

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The best geographic source of biocontrol agents is often assumed to be the region with the best climate match to the introduced range. However, in practice, it is often more convenient to collect from another location. As an experimental test of the importance of climate matching, we compared the performance of four North American sources of the planthopper, *Prokelisia marginata*, simultaneously introduced into each of five replicate sites in Willapa Bay, WA, as biological control agents for *Spartina alterniflora*. The sources were California, Georgia, Virginia and Rhode Island. The populations were monitored for 3 years. All four source populations established at most of the sites, but they exhibited significant differences in both reproduction during the summer and survival over winter. These differences were largely consistent with predictions from climate matching. The four populations also differed in spring emergence timing, adult wing morphology and age structure. Results of this study suggest that (1) the geographic source of biocontrol agents can affect performance and should be carefully chosen and (2) the use of multiple geographic sources of a biocontrol agent may improve overall performance in cases where biocontrol is applied in large target region spanning a range of climates.

# Effect of climate on biological control: a case study with diffuse knapweed in British Columbia, Canada

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With increasingly volatile and extreme weather patterns under global climate change, predicting how species and ecosystems will respond to these patterns is emerging as an important and rich area of ecological research. Climate mediates plant–insect interactions and consequently has the potential for positive or negative effects on biological control systems and the spread of invasive weeds. Observational evidence suggests that a recent dramatic reduction in the density of an invasive knapweed, *Centaurea diffusa*, in sites in the Okanagan Valley of British Columbia, Canada, is attributed to the biological agent *Larinus minutus*, a seed head weevil. This dramatic decline took place the same year in which the area experienced a severe late spring and summer drought. We have examined the effect of increased precipitation on the strength of the *Larinus*–*Centaurea* interaction. We conducted field experiments using constructed rain shelters and weekly watering treatments to assess the effectiveness of plant attack and seed destruction by *Larinus minutus* under moist and dry conditions. The results of these experiments suggest that insect attack is greater under dry conditions.

# The IRA and getting the result you want

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The current 'quagmire' of plant defence theories does little to assist the practitioners of classical biological control in the assessment, selection and interpretation of host feeding trials. The plethora of defence theories probably arose from a reductionist experimental approach across a wide variety of plant growth forms. Recent risk assessments of invasive species in forest ecosystems have offered a macroecological explanation of insect–plant interaction that is contrary to accepted plant defence hypotheses. The Island Resource Allocation (IRA) hypothesis provides a strong insight into how ecosystems function and as such can successfully predict the palatability of plants within genera, the metapopulation of species and plant life stages. The IRA offers some predictors for the likely outcome of classical biological control of target species in different ecosystems.

# Microclimate effects on biological control: water hyacinth in South Africa

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Low temperatures are repeatedly blamed for limiting the establishment of new biological control agents. Where successful establishment does take place, adequate control is often not achieved because of insufficient thermal accumulation. This scenario prevails in most water hyacinth biological control systems around South Africa. Microclimate was measured at 14 water hyacinth infestations distributed throughout South Africa's climatic regions. Water temperature, air temperature within the weed canopy and ambient air temperature were recorded at half-hour intervals for 24 months. Correlated with monthly field measures encompassing a variety of plant and insect parameters, this high-data resolution allowed for accurate descriptions of the seasonality and respective population dynamics prevalent in the system. Current literature on the thermal physiology of both *Neochetina* weevils, however, did not match their developmental pattern recorded in the field. Different climatic regions were found to be distinct in terms of both plant and insect phenology, population size, structure and growth rate, insect damage and therefore the subsequent levels of biological control achieved. Microclimate data provided a more accurate prediction of establishment that is otherwise too coarse when modelled on more broad scale climatic data.

# Habitat analysis of the rush skeleton weed root moth, *Bradyrrhoa gilveolella* (Lepidoptera: Pyralidae)

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The root feeding moth, *Bradyrrhoa gilveolella* (Treitschke) (Pyralidae) has been recently introduced into western United States for the control of rush skeleton weed, *Chondrilla juncea* L. (Asteraceae). Previous attempts to establish this moth in other countries, e.g. Australia and Argentina, have failed. Based on life history studies of the moth and habitat types at collection sites in Europe, we hypothesize that habitat will be a critical factor in successfully establishing the moth in North America. We surveyed 19 rush skeleton weed sites in northern Greece and southern Bulgaria, with and without populations of *Bradyrrhoa*. We compared these with release sites and potential release sites located in Idaho, USA. Multivariate analysis of site characteristics, vegetation and soil properties was used to investigate similarities amongst sites. Because of the low number of sites with the presence of *Bradyrrhoa*, it was difficult to discern distinct habitat differences. Soil texture appears to be the most important site factor common with sites with moth populations.

# Evaluating the performance of *Episimus utilis* (Lepidoptera: Tortricidae) on the invasive Brazilian peppertree in Florida

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Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), an introduced perennial tree from South America, has become widely established throughout central and south Florida because of its ability to invade a wide range of habitats from disturbed sites (e.g. along highways, canals) to natural communities (e.g. pinelands, mangrove forests). Genetic studies have identified two chloroplast DNA haplotypes of Brazilian peppertree in Florida that come from two genetically differentiated source populations in Brazil. Haplotype A is more common on the west coast of Florida, whereas haplotype B is more common on the east coast. In addition, hybridization between these two introduced populations has occurred extensively in Florida. A leaf roller from Brazil, *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae), has been selected as a potential biological control agent against Brazilian peppertree in Florida. The objectives of this study were to evaluate the performance of *E. utilis* on different Brazilian peppertree genotypes and plants subjected to different environmental conditions found in Florida (e.g. saline vs. fresh environments, soil fertility and soil moisture content). The ecological significance of the results is discussed in the context of predicting suitable sites for field releases to increase the possibility of establishment and subsequent effectiveness of this candidate biological control agent.

# Successful biological control of diffuse knapweed in British Columbia, Canada

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In the year of the first Biological Control of Weeds Symposium, the first introductions of insects were made in an attempt to control diffuse knapweed in Canada. In the next 23 years, 12 species of agents were introduced into Canada on this weed. Finally, beginning in 2000 and after the last insect species to be introduced, *Larinus minutus*, diffuse knapweed populations have declined dramatically in areas with high *Larinus* beetle densities. To determine if this decline was simply the result of drought, we have continued to monitor knapweed densities with the return of normal precipitation. Knapweed densities have not rebounded in areas with *L. minutus* but have resurged in areas without beetles and in the presence of other agents, *Urophora* spp. and *Sphenoptera jugoslavica*. To determine if attack by *L. minutus* is sufficient to reduce knapweed densities without other species of control agents, we have studied their impact in areas where knapweed has resurged after the eradication of all agents by wildfires. Cage experiments demonstrate the impacts of *L. minutus* on diffuse knapweed with and without *S. jugoslavica*. Preliminary results indicate a strong impact of *L. minutus* and their rapid reinvasion to newly infested knapweed sites.

# An integrated approach to invasive plant management: biocontrol and native plant interactions

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Native aquatic macrophytes are known as effective competitors against invasive aquatic plants. Yet, the invasive aquatic plants have an inherent competitive edge due to a lack of herbivores. Hence, the presence of diverse native plant assemblages coupled with sustained herbivory should hasten declines. To test this hypothesis in the aquatic environment, several experiments were conducted with and without plant competition and herbivory using insecticides to eliminate herbivores. In a 2-year study combining hydrilla biocontrol with native plant competition, overall tuber production was reduced 2-fold by native plant competition and 1.3-fold by herbivory alone. However, even more substantial decreases of more than fivefold were demonstrated when both were combined, stressing the importance of plant competition when found in conjunction with herbivory. Throughout this and other studies, native plants exhibited significant levels of damage due to invertebrate herbivory. However, little information is available that quantifies the impact of herbivores on native plants. In a second study examining native herbivore impacts to submersed and floating leaf species, only the two floating-leaved species, *Potamogeton nodosus* and *Potamogeton illinoensis* exhibited a 37% and 72% decrease in biomass in the presence of herbivory, respectively. Hence, competitive edge in native plants is apparently curtailed significantly because of herbivory.

# Impact of host-plant water stress on the interaction between *Mecinus janthinus* and *Linaria dalmatica*

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*Mecinus janthinus* (Coleoptera: Curculionidae) is a stem-boring weevil that has been introduced as a biological control agent for Dalmatian toadflax (*Linaria dalmatica* L). We examined the impact of host-plant water stress on plant performance, weevil choice, weevil oviposition rates and survival. Weevils preferred well-watered plants and plants previously attacked by other females. The weevil had greatest impact on toadflax fruit production in high-water treatment plants but had greatest impact on stem production and plant weight in the intermediate water treatment. None of the plant performance variables measured significantly responded to weevil feeding in the low-water treatment. However, beetle survivorship increased with increasing water supplied to the plant. These results indicate that the impact of this biological control agent on its host plant is context-dependent and varies with the amount of water available to the plant. *Mecinus* prefers and performs better in plants that receive more water, yet these plants are also less susceptible to weevil attack. These data suggest that studies of plant quality and herbivore impact need to include herbivore preference and performance to generate a complete picture of the interaction.

# Impact of insect herbivory on dispersal in *Hydrilla verticillata* (L.f.) Royle

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*Hydrilla* is an invasive aquatic plant that spreads through a variety of vegetative structures. Fragments, long-distance dispersal and vegetative reproductive propagules, increase the ability of *Hydrilla* to colonize new and distant locations. Sustained levels of herbivory by leaf-mining flies (*Hydrellia pakistanae* and *Hydrellia balciunasi*) can reduce biomass and impact the ability of hydrilla to photosynthesize. Impacts from herbivory apparently can increase fragmentation at points of feeding. If greater fragmentation occurs because of increased fly damage, how viable are these fragments? To answer this question, viability of fragments with low (0–30%), medium (40–60%) and high (70–100%) leaf damage were compared. Fragments with high levels of leaf damage produced three times less biomass when compared with hydrilla fragments with low leaf damage. Fragment establishment was also studied based on settling, rooting and anchoring success of individual fragments. Results indicate that no highly damaged fragments settled, produced roots or anchored. However, more than 80% of the control fragments produced roots and anchored, as compared with only 20% by low- and medium-damaged fragments. Herbivory apparently has great impact, significantly reducing fragment viability.

# Dynamics of invasive plant monocultures after the establishment of natural enemies: an example from the *Melaleuca quinquenervia* system in Florida

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*Melaleuca quinquenervia* (melaleuca), a native tree of Australian origin, has become one of the most invasive plants in Florida. Biological control was implemented as a long-term solution to melaleuca control in Florida. Now several natural enemies (insects and a pathogen) of melaleuca are well-established in Florida. Their impact on melaleuca populations is being monitored for several years. Insect–fungus integration in field conditions showed significant impact on stump regrowth control of melaleuca. During the period when natural enemies prevailed, melaleuca stand density and basal area declined at a greater rate across study sites. *Myrtaceae* (represented by melaleuca) and *Cyperaceae* (represented by saw grass, *Cladium jamaicense*) were the first and second most important families during 1997, respectively. Their family importance values were reduced significantly during 2004/05, with the increase of the importance values of other families in the stand. Overall plant richness and diversity in melaleuca stands was higher (compared with 1997) in 2004/05. Within stands, plant species richness and diversity indices were relatively higher at interior than at peripheral sections. Melaleuca-vacated spaces in the stands were colonized mostly by native plant species. This phenomenon is predicted to intensify as the natural enemies continue to impact melaleuca monocultures in Florida.

# Modelling of *Diorhabda elongata* dispersal during the initial stages of establishment for the control of *Tamarix* spp.

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Critical questions associated with use of *Diorhabda* for control of *Tamarix* are the following: How far and how fast will the beetle defoliate salt cedar trees after the insect's release? What are the factors that affect the dispersal of the insect and the salt cedar defoliation? One effective way to answer these questions is modelling dispersal of the beetle and the defoliation that it causes. Modelling strategies depend on whether the *Diorhabda* is in the initial stages of establishment or in later stages when it is already well-established. The diffusion model developed by Kovalev was fit to observed data at Big Spring, TX, in 2005 and 2006. Kovalev's model was able to fit satisfactorily the data of larval density. When Kovalev's waves are not symmetric, it is mainly because of unequal distances between transect quadrants, which happens in areas of nonuniformly distributed salt cedar. The modelled larval dispersal front reached 100 m in transect 2 during 2005 and 510 m in 2006. The defoliation front, associated with the high density of larvae, reached between 60 and 80 m in 2005 and about 240 m in 2006. Fitting statistical models to data collected is in progress.

# Seed feeders: why do so few work and can we improve our selection decisions?

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Seed feeders have a mixed record in weed biological control. They are typically easy to test, rear, release and establish and can be particularly useful when targeting beneficial plants. However, seed predation rates are often relatively low. Also, evaluating the impacts of seed predation on the population dynamics of the plant, particularly on rates of spread, is difficult. In this paper, we present the results from the field assessment and modelling of a seed-feeding bruchid (*Penthobruchus germaini*) on the woody legume *Parkinsonia aculeata*. The seed feeder is typically abundant, and both the target and the seed feeder occur across diverse climate zone (from the wet–dry tropics to the arid interior) and habitats (including upland and wetlands). In particular, we (1) determine seed predation rates and identify factors that were limiting seed predation rates and impacts through national field surveys and (2) estimate the effect of seed predation on rates of population increase (using DYMEX, a simulation population model) and rates of spread (using a cellular automata model). The modelling work and field evaluations both highlighted general factors that may limit the impact of seed feeders on plant populations. We discuss whether agent selection decisions involving seed-feeding insects can be improved to identify potential agents that will have the greatest impact and avoid releasing ineffective agents.

# Theme 2:

# Benefit/Risk—Cost Analyses

## Session Chair: Ernest (Del) Delfosse

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Keynote Presenter

# Return on investment: determining the economic impact of biological control programmes

R. McFadyen[1]

*Summary*

In >100 years of weed biological control, few economic impact assessments of biological control programmes have been undertaken, and all were successes. Yet biological control is still largely paid for by governments, who need proof of the return on their investment. Cost/benefit analyses can also be used to rank biological control against other management methods. A recent economic impact assessment of all weed biological control undertaken in Australia since 1903, including both successes and failures, demonstrated annual benefits of $95.3 million from an average annual investment of $4.3 million (Aus$, 2005 values), a cost/benefit ratio of 23:1. Even with the enormous economic impact of the prickly pear success excluded, the cost/benefit ratio of all other programmes was 12:1. The benefit came from 17 successful programmes: two, which are usually considered failures, in fact returned strongly positive benefits because small reductions in the weed problem nevertheless resulted in considerable cost savings.

The scarcity of economic studies has many causes: long period from commencement to full field results; difficulties in assigning monetary values to biodiversity and social impacts; and difficulties in assessing impacts of biological control. The Australian study demonstrated the economic returns from partial successes, where these reduce the costs of other management methods. It also demonstrated the importance of obtaining baseline economic data before starting biological control and at intervals during the agent release period. Seeking advice from economists at all stages of a biological control programme must become as routine as consulting statisticians.

***Keywords***: weed biological control, cost/benefit, success rates, Australia.

## Introduction

A check of the proceedings of the last three international symposia, plus the main biological control journals, confirmed that although many evaluation studies have been published, few included any economic data. Doeleman (1990) on the biological control programme against salvinia *Salvinia molesta* DS Mitchell and Jarvis *et al.* (2006) on scotch broom *Cytisus scoparius* (L.) Link were among the few exceptions. Dhileepan (2003) listed published evaluation studies from Australia, of which 32 reported impacts at plant level and 20 reported impacts at plant population level, but only three analysed the economic impact.

The 1996 Symposium had a session on *Evaluation and Economics* with three papers, of which only one (Coombs *et al.*, 1996) analysed the economic benefits, while the 2000 Bozeman Symposium had no papers on economic analysis. The 2003 Symposium session on *Risk Analysis* treated this purely as biological risks with no mention of economic risks. However, Stanley and Fowler (2004) stated that economic analyses are an ‘important part of resolving conflicts of interests’ and ‘Decision-makers often find arguments couched in monetary terms to be more convincing’. The theme *Evaluation* included a report of a long-term study showing massive reduction in thistles in rangelands in the western United States (Kok *et al.*, 2004), and other papers demonstrating impact on weed populations, but

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with no economic data. The only economic assessment was of the control of the waterweed *Azolla filiculoides* Lamarck in South Africa (McConnachie *et al.*, 2003).

A January 2004 conference in the United States discussed 'benefits and risks of biological control' in order to 'address critical issues facing policy makers'. However, no economic benefit/risk analyses or consideration of return on investment were included. There were 20 papers dealing with risks and how to predict and reduce these, but only one on the 'economic framework for decision-making in biocontrol' (Jetter, 2005). In summary, although there is an increasing recognition of the need to demonstrate impact on weed populations and native biodiversity (e.g. Coombs *et al.*, 2004; Story *et al.*, 2006; Barton *et al.*, 2007), economic impact analyses remain very much the exception.

## Value of economic impact assessment

Most weed biological control is funded by public money in one form or another and thus competes for funding with other government programmes. The demonstrated cost-effectiveness of a control method is a reason for continuing or increasing both the use of the method and the resources dedicated to it (Culliney, 2005; Lodge *et al.*, 2006). For example, net economic benefits from pre-introduction screening of plant imports is a justification both for major policy change in the United States and for the maintenance of expensive border controls in Australia (Keller *et al.*, 2007), and McConnachie *et al.* (2003) highlighted the use of cost/benefit analyses to rank biological control against other means of control.

Syrett, Briese and Hoffmann (2000), in their excellent overall summary of economic evaluation in weed biological control, make several important and still-valid statements. For example, it is 'important to know how successful the technique is overall. If a high proportion of programmes are successful, then the likelihood of a new programme being successful is relatively high too.' Jetter (2005) pointed out that policy decisions are based on economic criteria, which in turn depend on the probability of success. Increasingly, risk assessment (or risk–benefit–cost assessment) is being applied worldwide to most activities. This requires identification of hazard and benefits, and then exposure analysis (quantitative assessment of probabilities/likelihood) (Sheppard *et al.*, 2003). It is therefore essential to know the probability that the programme will result in a positive economic outcome (i.e. economic return will exceed economic costs), which allows the calculation of the risk-weighted return of an individual investment. This can then be used to prioritise investments across the entire portfolio. Note that this is not probability of 'complete' success but the probability of a positive return on investment.

In any risk assessment, probabilities are calculated from historic data for similar activities. For this reason, it is important to include failures in the economic analysis, as only this can give real estimates of success rates while also including benefits from partial successes, which otherwise may be seriously undervalued. For weed biological control, there is therefore a need to measure all research and research-related costs from all programmes undertaken, and to assess this against all benefits gained. A similar analysis was undertaken for all programmes of the Australian Centre for International Agricultural Research (Raitzer and Lindner, 2005), and could be done for weed biological control on a country or state basis. Only in this way can we calculate the true probability of a positive return on investment. 'Selected' case studies where failures are not included (van Wilgen *et al.*, 2004) are useful but do not help assess the probability of failure.

This overall approach will always underestimate benefits, because the costs are already realised and are comparatively easy to determine, while benefits can only be calculated where both the baseline data to measure change has been collected and the impact assessments have been done. Benefits may also continue to increase after the assessment period; hence, the approach is inherently conservative.

# Economic impact assessment of Australian weed biological control programmes

This section is a summary of results from the publication by Page and Lacey (2006) of the consulting firm AEC Group Ltd. Their study aimed to determine the cost of all biological control research undertaken in Australia since the first prickly pear programme in 1903, and compare this against the total benefits received. The work was funded by the Cooperative Research Centre for Australian Weed Management (Weeds CRC) as part of our Biological Control Evaluation project. Data were collected by Colin Wilson, with assistance from many Weeds CRC staff. The direct cost of this project was Aus$85,000 plus many uncosted hours of scientist time.

## Methods

Only economic costs and benefits were considered: other benefits were listed but not included in the analysis. Costs of current programmes were excluded where it was still too early for field impacts to be realised. However, completed programmes where no agents were released or established were counted as failures and the costs included in the analysis.

The first step was to assemble previously undertaken economic analyses and convert these to current values. All economic data throughout the study were converted to and are cited as 2005 Aus$ values. Available *ex-ante* cost–benefit analyses were recalculated in the light of actual results. The next step was to list all weed biolog-

ical control programmes undertaken, including those that did not lead to releases, and assemble cost data wherever possible. Data on the costs of research and releases were usually available from internal or published reports and records. Where data were unavailable or incomplete, the duration of the research in years and the number of staff employed (usually known from reports) was used to calculate costs, using a factor of Aus$300,000 per scientist-year whether employed in Australia or overseas.

The most difficult part proved to be locating data on the economic impacts of the weeds prior to biological control. Despite careful searches of the literature, including the original reports used to justify biological control programmes, all too often there was little or no data on the value of losses due to the weed or on the cost of control, and little quantitative information on the extent of the weed or the rate of spread. However, all available information was collated and converted to 2005 Aus$ values. Independent economic data from the Australian Bureau of Statistics (ABS) Australian Farm Surveys were used whenever available, but ABS surveys did not include questions on the cost of weeds until 2006; therefore, comparable national data was not available.

The biological control programmes against the three floating water weeds salvinia, water hyacinth *Eichhornia crassipes* (Mart.) and water lettuce *Pistia stratiotes* L. were considered as one programme, with both costs and benefits summed into one amount. These weeds occupy the same water surfaces and each is able, if not controlled, to occupy 100% of water surfaces. Therefore, the benefits only accrue if all three are simultaneously controlled, which was achieved between 1975 and 1995.

The long and expensive programme on Noogoora burr (*Xanthium occidentale* Bertol.), with overseas exploration from 1930 to 1970, resulted in the establishment of three insects but no impact on the weed. A rust disease *Puccinia xanthii* Schw. was studied in France but not introduced on the grounds that it was insufficiently host-specific. In 1974 the same rust turned up in Brisbane and in north Queensland (Morin *et al.*, 1996), almost certainly illegally imported by landholders in contact with the research group. The rust spread rapidly and, together with a moth released against parthenium weed (*Parthenium hysterophorus* L.), has given almost complete control of Noogoora burr over most of the affected lands. As the introduction of the rust, although illegal, was a direct consequence of the biological control investigations, the benefits have been included, as economically damaging consequences, whether intended or not, have also been included in all analyses.

Information on the biological control impacts was obtained from publications, unpublished reports and personal communications from scientists involved in the projects. Proper evaluations had not been undertaken for most programmes, even for successful ones. Where agents had established, information on spread and impact was often very incomplete, as was information on impact on the weed population. Too often, there was only expert opinion, expressed in percent reduction of the weed population, percent of the total area of weed infestation affected, and percent of years with maximal impact.

## Results

Results are presented in Table 1 (summary of Table 3.1 in Page and Lacey, 2006). A total of 36 weed biological control programmes in Australia have been undertaken in the 100 years to 2004, but for three of these (against crofton weed *Ageratina adenophora* (Sprengel), St John's wort *Hypericum perforatum* L. and docks *Rumex* spp.), although the biological control has been largely successful, key data on costs or benefits could not be obtained. There were existing economic cost/benefit analyses for eight programmes (e.g. Adamson and Bray, 1999), though some (e.g. Nordblom *et al.*, 2002) were *ex-ante* studies, and actual realised benefits had never been assessed.

Of the 33 programmes with economic data, no agents were ever released or established for five of these. Economic benefits were zero in a further four where established agents have, to date, proven ineffective. All remaining programmes returned economic benefits. In three (alligator weed *Alternanthera philoxeroides* (Martius), groundsel bush *Baccharis halimifolia* L. and *Sida* spp.), although successful control of the weed was achieved, the benefit/cost ratio was less than 1 because the direct economic impact of the weed was small. For some others where the benefit/cost ratio is <1, the benefits are still increasing and the assessments may be premature.

Seventeen programmes out of the 33 assessed returned a positive return on investment; i.e. economic benefits exceeded costs. Thirteen of these have resulted in very large economic benefits—the prickly pear (*Opuntia* spp.) programme, but also those against ragweed *Ambrosia artemisiifolia* L., nodding thistle *Carduus nutans* L., skeleton weed *Chondrilla juncea* L., rubber vine *Cryptostegia grandiflora* R. Br., Paterson's curse *Echium plantagineum* L., harrisia cactus *Harrisia martinii* (Labouret), giant sensitive plant *Mimosa diplotricha* C. Wright, *Onopordum* thistles, parthenium weed, the three water weeds, ragwort *Senecio jacobaea* L., and Noogoora burr. Surprisingly, the programmes against lantana *Lantana camara* L. and blackberry *Rubus fruticosus* L. agg., both generally considered to be still unsuccessful, returned positive cost/benefit ratios because the economic losses from these two weeds are so great that relatively small reductions in these losses are worth a great deal of money. On the other hand, the successful control of bridal creeper *Asparagus asparagoides* (L.) did not result in large economic gains because it is almost entirely

**Table 1.** Total costs (converted to Aus$ million 2005 values), benefits (net present value, Aus$ million), and benefit/cost ratios for 33 Australian weed biological control programmes.

| Weed species | Yrs | Cost | Benefits NPV | Benefit /cost | Comment |
|---|---|---|---|---|---|
| *Senecio madagascariensis* (fireweed) | 1990–1994 | 0.4 | 0 | 0 | No agents released |
| *Senna obtusifolia* (sicklepod) | 1992–2000 | 0.7 | 0 | 0 | No agents released |
| *Emex australis* & *E. spinosa* (spiny emex/double gee) | 1974–1978 | 2.0 | 0 | 0 | No agents established |
| *Heliotropium europaeum* (common heliotrope) | 1973–1991 | 4.4 | 0 | 0 | No agents established |
| *Lantana montevidensis* (creeping lantana) | 1996–2000 | 0.2 | 0 | 0 | No agents established |
| *Acacia nilotica* (prickly acacia) | 1980 → | 5.3 | 0 | 0 | Agents established but ineffective |
| *Ageratina riparia* (mist-flower) | 1986–2001 | 0.2 | 0 | 0 | Agent established but ineffective |
| *Parkinsonia aculeata* (parkinsonia) | 1983–1990 | 1.6 | 0 | 0 | Agents established but largely ineffective |
| *Silybum marianum* & *Cirsium vulgare* (variegated and spear thistle) | 1988–2002 | 3.0 | 0 | 0 | Agents established but ineffective |
| *Marrubium vulgare* (horehound) | 1989–2001 | 1.8 | 0.3 | 0.2 | Benefits still increasing |
| *Alternanthera philoxeroides* (alligator weed) | 1976–2004 | 1.4 | 0.3 | 0.4 | Social benefits not costed |
| *Chrysanthemoides monilifera* (bitou bush/bone seed) | 1990 → | 7.1 | 2.7 | 0.5 | Agents established but largely ineffective |
| *Prosopis* spp. (mesquite) | 1992–2004 | 2.3 | 0.8 | 0.5 | Benefits still increasing |
| *Sida acuta* & *S. rhombifolia* (Paddy's lucerne) | 1984–1999 | 4.2 | 1.1 | 0.5 | |
| *Baccharis halimifolia* (groundsel bush) | 1961–1998 | 9.6 | 2.1 | 0.7 | Changed land use reduced benefits |
| *Mimosa pigra* (mimosa) | 1981– 2004 | 21.6 | 6.1 | 0.8 | Benefits still increasing |
| *Asparagus asparagoides* (bridal creeper) | 1990– 2004 | 7.3 | 8.2 | 2.0 | Very large biodiversity benefits |
| *Rubus fruticosus* agg. (blackberry) | 1977–2004 | 4.9 | 6.1 | 2.5 | Benefits still increasing |
| *Xanthium occidentale* (Noogoora burr) | 1930–1975 | 10.1 | 23.4 | n/a | Data from earlier study |
| *Lantana camara* (lantana) | 1914–2004 | 13.6 | 3.0 | 5.6 | Benefits largely from first agents |
| *Carduus nutans* (nodding thistle) | 1986–2000 | | 81.3 | 6.9 | Data from earlier study |
| *Parthenium hysterophorus* (parthenium weed) | 1977–2004 | 11.0 | 38.6 | 7.2 | Benefits still increasing |
| *Onopordum* spp. (Scotch, stemless & Illyrian thistles) | 1988–2004 | 3.7 | 20.1 | 9.6 | |
| *Carduus pycnocephalus* & *C. tenuiflora* (slender thistles) | 1987–1997 | 2.1 | 22.5 | 14.1 | |
| *Mimosa diplotricha* (giant sensitive plant) | 1982–1992 | 1.7 | 21.4 | 18 | |
| *Harrisia* spp. (Harrisia cactus) | 1959–1976 | 1.0 | 19.4 | 23.5 | Additional uncosted biodiversity benefits |
| *Salvinia molesta, Eichornia crassipes, Pistia stratiotes* (waterweeds) | 1974–1993 | 5.1 | 79.4 | 27.5 | |
| *Senecio jacobaea* (ragwort) | 1977–2004 | 7.9 | 97.2 | 32.4 | Benefits still increasing |
| *Echium plantagineum* (Paterson's curse) | 1972–2004 | | 1,201.3 | 52.0 | Data from earlier study |

**Table 1.** (Continued) Total costs (converted to Aus$ million 2005 values), benefits (net present value, Aus$ million), and benefit/cost ratios for 33 Australian weed biological control programmes.

| Weed species | Yrs | Cost | Benefits NPV | Benefit /cost | Comment |
|---|---|---|---|---|---|
| *Ambrosia artemisiifolia* (annual ragweed) | 1985–1991 | 0.6 | 52.5 | 103.7 | |
| *Cryptostegia grandiflora* (rubber vine) | 1984–2004 | 3.6 | 234.6 | 108.8 | Additional uncosted biodiversity benefits |
| *Chondrilla juncea* (skeleton weed) | | | 1,425 | 112.1 | Data from earlier study |
| *Opuntia* spp. (prickly pears) | 1903–1987 | 21.1 | 3,110.3 | 312.3 | |

Shaded data are from earlier studies, not included in overall benefit/cost ratio.
Three programmes—*Ageratina adenophora* (crofton weed), *Hypericum perforatum* (St John's wort), *Rumex* spp. (docks)—were excluded for lack of economic data, but all resulted in significant control of the weed.

a weed of natural ecosystems and the direct financial costs of the weed are small.

Costs also varied greatly. Some programmes continued over decades, with years of overseas research and the employment of several scientists; the most expensive being that against *Mimosa pigra* L., with a total cost of Aus$21.6 million. The cheapest successful programme was against annual ragweed at a cost of Aus$0.6 million. This cost was low because the successful agents were imported for the control of the closely related parthenium weed, and the only additional costs were for extra releases in ragweed areas. Two other insects from the US were tested, but this was undertaken alongside another major project and costs were minimal. However, median cost for the 17 successful programmes (including bridal creeper, groundsel bush, and blackberry) was Aus$7 million and the duration 14 to 27 years. It is unrealistic to expect good results for smaller investments (cf Coombs *et al.*, 2004).

As was expected, benefits from the control of the prickly pears were enormous. For simplicity, only data from the statistical area of the Darling Downs in southern Queensland were used in the calculations. This is a large and rich agricultural area which was almost totally unusable due to prickly pear until after the impact of cactoblastis in the early 1930s. Benefits from infested land in central and north Queensland and in New South Wales have not been included; their inclusion might double the measured economic return. In the calculations, economic benefits from land under cultivation were considered to have ceased in the 1960s because the larger machines available from the 1950s can handle even land densely infested with prickly pear. However, in land used for grazing, there is still no other economic control for prickly pears. Social and medical benefits from reduction in injuries and infections due to spines were not considered.

The overall benefit/cost ratio was 23.1 for the 28 programmes where data could be analysed—an astonishing result. Even if the iconic prickly pear success is excluded, the overall benefit/cost ratio is 12.3. Out of the total 36 programmes (including those where economic analysis was not possible), only nine were failures, with few or no economic benefits.

## Issues for the future

There are two major messages from this study: the large benefits from even partial control of major and widespread weeds such as lantana and blackberry, and the overwhelming importance of documenting the economic costs of the target weed at the start of a biological control programme. The key issue is to quantify the economic costs of the target weed at the start, so that the benefits from any reduction in its abundance can in turn be quantified.

Therefore, the first step, unfortunately often omitted, is a good economic assessment of the economic losses caused by the weed; e.g. for *Melaleuca quinquenervia* (Cav.) in Florida (Diamond and Davies, 1991; Turner *et al.*, 1998) and for *Tamarix* spp. in the United States [Zavaleta (2000) and reviews by Culliney (2005) and Coombs *et al.* (2004)]. Ideally, this information should be part of an *ex-ante* benefit/cost study prior to starting any biological control programme. This is best done by independent economists and made part of the decision process (Greer and Sheppard, 1990; Jarvis *et al.*, 2006). Key principles are transparency; i.e. key assumptions, data sources, and data treatment must be clearly described and explained; and analytical rigour in the use of the data (Raitzer and Lindner, 2005). If the cost basis used is made explicit, studies can be critiqued by others and future updating undertaken. For example, a study on the productivity benefits from parthenium biological control (Adamson and Bray, 1999) used a range of 80 to 120 cents per kg for the price of cattle. Within three years, market prices more than doubled to 200 to 400 cents per kg, and the economic benefits increased accordingly.

As part of an *ex-ante* analysis, the question must be asked: 'If the target weed is removed, would yield losses/weed control costs be reduced?' If the target weed would be replaced by other weeds and control costs would not fall, then a biological control programme

should not be started. If, however, control of the replacement weeds would be cheaper or yield losses be reduced, there would still be an economic benefit. For example, in Indonesia and elsewhere, the weed chromolaena, *Chromolaena odorata* (L.), often replaced other weeds including lantana, which then return once chromolaena has been controlled. However, control costs (or production losses) for chromolaena greatly exceed those from lantana; hence, there is still a benefit from its successful biological control even if lantana subsequently re-invades. Other variables can also influence costs; for example, chromolaena is a weed in oil palm plantations in both Indonesia and Papua New Guinea, but in Indonesia, the oil palms are much more densely planted so that the weed is shaded out in mature plantations and control is only needed for five years. Costs are therefore much greater in Papua New Guinea where control is needed for the full 25-year life of the plantation. With weedy trees in South Africa, 70% of the losses are due to reductions in water flows (van Wilgen *et al.*, 2004), which would not apply in other countries with different native floras or different hydrology.

## Quick and dirty or 'scientific'?

"There is often a painful choice in…economic analyses…one may choose only two among the three characteristics: fast, accurate and cheap!" (T. Nordblom, 2006, unpublished paper). The decision has to be based on the objective – to produce scientific papers in the highest-impact journals, or to convince policy makers and national governments? A recent discussion on the assessment of augmentative biological control (used in greenhouse and other horticultural crops) deals with exactly this problem—whether it is necessary to have 'scientifically valid studies' or whether reduced industry use of pesticides is sufficient (van Lenteren, 2006; Collier and Steenwyk, 2006). To quote: "(the issue is) what represents convincing evidence", "In our view, the type of evidence that makes a convincing case for efficacy…is quantitative data from replicated field experiments with valid control plots. The data should also be published in a peer-reviewed journal instead of the "grey" literature…" and "implementation [*by farmers*] is not equivalent to quantitative data from scientifically rigorous studies." (Collier and Steenwyk, 2006 p.120).

However, economic analyses for policy purposes are rarely, if ever, published in peer-reviewed scientific journals. The recent Stern Review 'The Economics of Climate Change' first appeared as a government document in 2006, then on their website with comments and postscript reports, and in 2007 was published as hardcopy by Cambridge University Press. For a more relevant, less-prestigious example, the Raitzer and Lindner (2005) review was published by ACIAR in their Impact Assessment Series, put on their website, and is obtainable only from them.

A related issue is the level of proof required. Detailed scientific studies can be used to demonstrate impact of agents in small-scale studies (e.g. Dhileepan, 2001) but this does not demonstrate landscape-scale reduction in weed impacts. Alternatively, independent economic measures, or properly conducted end-user surveys (e.g. Ireson *et al.*, 2007) may demonstrate cost reductions over several years, but with only correlative evidence that this is due to the impacts of biological control. Ideally, there should be both: detailed in-field evaluations using agent exclusion methods on plots planted to obtain pre-control levels of the weed, demonstrating increased production and/or reduced control costs, and supported by independent evidence of end-user cost savings.

However, it is equally acceptable, and much cheaper, to start with end-user results, such as the reduction in livestock deaths used by Coombs *et al.* (1996) in their assessment of the impact of the ragwort biological control programme in Oregon. They used the dramatic fall over a 20-year period in livestock deaths from pyrrolizidine alkaloid poisoning at local veterinary laboratories as a measure of benefits. Data for the estimate of programme costs were largely available. They calculated a 15:1 benefit cost ratio at 7% discount, and an annual benefit of $5 million. Correlative analysis based on industry-wide data is widely accepted as scientifically valid for other policy decisions such as health interventions (e.g. Productivity Commission, 2005), and it is time to recognise that it is equally valid for biological control.

# Conclusion

If the overall objective of a weed biological control programme is to reduce the weed's harmful impact, then Key Performance Indicators (KPI) will measure the degree to which this has been achieved, and assessment protocols must be designed to measure these KPIs. This means the initial state prior to the biological control programme must be adequately recorded. For most weeds, even nonproduction impacts have an economic aspect—if control was cheap or easy, the community would not permit weeds to take over environmental areas. It is the economic cost that makes removal and restoration unviable except for very small areas.

Economic impact is the sum of many factors: loss of agricultural productivity; extent of infestation, actual and potential; spread rate; cost of removal; and frequency of recurrence. Measurement of these at the start, even if only on a coarse scale, is essential to make future assessments possible. This is most easily achieved by developing an *ex-ante* cost–benefit study for each programme (Coombs *et al.*, 2004). Such analyses immediately clarify where data are not available, as well as identifying the critical outcomes desired. In a long programme, the analysis can be repeated after several years, when more information is available (e.g. whether

suitable agents can be found and established; spread rate of weed). Comparison over several years may identify significant gains even from 'unsuccessful' programmes (Hoffmann and Moran, in these proceedings). *Ex-ante* studies, using realistic probabilities of success based on historic rates for the country and type of weed, and taking into account the full range of potential costs and benefits, are powerful tools to convince funding agencies. In time, *ex-post* analyses based on these data will clarify the true probabilities of failure, and consequently, return-on-investment for weed biological control. 'Halfway' benefit/cost analysis, when agent spread and impact are known, can also help decide whether to spend more resources on increased releases to achieve faster results (Nordbloom *et al.*, 2002).

The key messages from our study, therefore, were: biological control is a very cost-effective method and has given excellent returns-on-investment for Australian governments; indeed, the only better investment in weed management is expenditure to prevent new incursions; and organizations and governments undertaking biological control need to ensure adequate economic data is collected at the start of the programmes and at intervals throughout.

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# Post-release non-target monitoring of *Mogulones cruciger*, a biological control agent released to control *Cynoglossum officinale* in Canada

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*Summary*

Non-target effects of approved biological control agents have raised questions about the safety of biological control of weeds and resulted in an increased emphasis on monitoring and reporting of non-target effects as part of post-release assessments. This is particularly important in the case of the root-mining weevil *Mogulones cruciger* (Herbst), which was approved in Canada to control houndstongue, *Cynoglossum officinale* L., but not in the United States because of concerns over its environmental safety. To address these concerns and the potential for non-target effects, we monitored co-occurring confamilial Boraginaceae species at six *M. cruciger* release sites in Alberta and British Columbia over two years. All four co-occurring species were attacked by the weevil to varying degrees although attack was inconsistent between years and sites, and non-targets were mostly attacked to a lesser degree than houndstongue. There was a positive relationship between the probability of non-target attack and houndstongue attack rate by *M. cruciger* indicating potential spillovers and early evidence suggests non-target attack may be transitory. The comparison between the pre- and post-release evaluations and preliminary plant volatile, electroantennogram, and host-choice behaviour data suggest that chemical ecology may provide an important tool in understanding an insect's host-choice selection in pre-release host-specificity assessments.

***Keywords***: houndstongue, non-target effects, host-choice behaviour, chemical ecology

## Introduction

Examples of non-target effects have created widespread interest and concern through both the scientific and public communities about the environmental safety of biological weed control agents (Simberloff and Stiling, 1996; Louda *et al.*, 1997, 2003 a, b; Strong, 1997; Thomas and Willis, 1998; Pemberton, 2000). The predictability of an agent's host range is often at the center of the debate. Conventional host-specificity tests are used to determine an insect species' physiological and ecological host range, which is then used to predict an insect's realized host range once released in the invaded range (Schaffner, 2001; van Klinken and Edwards, 2002). Studies demonstrate that the physiological host range, which can be reliably determined experimentally (e.g. Papaj and Rausher, 1983; Szentesi and Jermy, 1990; van Klinken, 2000; van Klinken and Heard, 2000), appears to be an effective criterion for identifying species at risk of attack by introduced agents, since there is no example of an insect agent attacking a plant outside its physiological host range (Fowler *et al.*, 2000; Pemberton, 2000; van Klinken and Edwards, 2002). A species' realized host range, however, is almost certainly narrower than its physiological host range, which is evident from the narrowing host range that is frequently observed under increasingly natural testing conditions (i.e. multiple-choice tests and open-field tests). However, adequate pre-release screening protocols to

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predict this have not been developed (Van Driesche *et al.*, 2000; Schaffner, 2001; Hopper, 2001; and references therein). Realized host ranges will be limited due to phenological, ecological, and behavioural constraints. While phenological and ecological factors likely differ among continents and habitats, behavioural factors are inherent to the agent and could be examined as part of improved pre-release assessment protocols.

The host selection process of phytophagous insects typically progresses from host finding (dependent upon volatile and visual cues), through host examination (dependent in addition on gustatory and tactile cues), to host acceptance (oviposition or sustained feeding) (Dethier, 1982; Miller and Strickler, 1984; Bernays and Chapman, 1994). Each stage is dependent upon completion of the previous stage. Thus, in nature, host examination and acceptance, as evaluated in no-choice bioassays, cannot occur unless the insect arrives at the potential non-target host during the host-finding stage. Therefore, knowledge of the chemical basis for host-finding behaviour might improve our ability to assess whether a non-target plant species that can support the development of a biocontrol agent would be at risk of attack in the field (Thomas and Willis, 1998; Heard, 2000; Schaffner, 2001).

The *Mogulones cruciger* Herbst, houndstongue (*Cynoglossum officinale* L.), system provides a prime example of the importance of such testing. *Mogulones cruciger* is a root-feeding weevil that has been released in Canada to control the noxious rangeland weed, houndstongue. Release in the United States, however, has been denied because of concerns about its environmental safety. Previous host-specificity testing has demonstrated that *M. cruciger*'s physiological host range is fairly broad, but it has always shown a strong preference for houndstongue (Jordan *et al.*, 1993; De Clerck-Floate *et al.*, 1996; De Clerck-Floate and Schwarzlaender, 2002; Andreas, 2004). A recent study of six field sites in Canada found that *M. cruciger* was utilizing four confamilial species growing sympatrically with houndstongue (Andreas, 2004). The attacked Boraginaceae species are *Cryptantha spiculifera* (Eastw.) Payson, *Hackelia floribunda* (Lehm.) I.M. Johnston, *Lappula squarrosa* (Retz.) Dumort and *Lithospermum ruderale* Dougl. ex Lehm.. The first three species are within the known physiological host range of *M. cruciger* (De Clerck-Floate *et al.*, 1996; De Clerck-Floate and Schwarzlaender, 2002; Andreas, 2004) while the latter species has not been sufficiently tested because its cultivation has not been successful. Because *M. cruciger* is known to prefer houndstongue (Jordan *et al.*, 1993; De Clerck-Floate *et al.*, 1996; De Clerck-Floate and Schwarzlaender, 2002; Andreas, 2004), we hypothesize that these non-target attacks result from 'spillover', 'sensitization/central excitation' effects or both. In spillover, high population densities on the target result in some insects colonizing non-targets due to random dispersal especially after the target resource begins to be depleted (Strong, 1997). In sensitization/central excitation, acceptance thresholds for non-targets are lowered after contact with the true host or in the presence of ambient volatile organic compounds emitted by the true host (Marohasy, 1996, 1998; Withers and Barton Browne, 1998). Regardless of the specific mechanism, these recorded non-target attacks indicate a need to determine the risk that *M. cruciger* poses to native species that are within the physiological host range and within the potential range of the weevil's dispersal from target host populations. Therefore, we explored the early stages of host-selection behaviour and its underlying phytochemical basis to determine the plant species likely to be colonized by *M. cruciger*.

# Materials and methods

## Study organisms

Houndstongue is a Eurasian herbaceous, facultative, short-lived perennial. Plants produce rosettes in the first year and typically reproduce in the second or third year (Wesselingh *et al.*, 1997). After sexual reproduction, barbed nutlets are formed and dispersed via epizoochory (De Clerck-Floate, 1997). In Europe, the plant is found in sand dunes, roadsides, and open woodlands (Tansley and Adamson, 1925; Tutin *et al.*, 1972; Hegi, 1975; Klinkhamer and de Jong, 1988). In North America, this ruderal species colonizes disturbed areas, rangelands, pastures, and forests (Macoun, 1884; Upadhyaya and Cranston, 1991).

*Mogulones cruciger* is a root-feeding weevil native to central Europe. In spring, after hibernation, adults occur on above-ground plant parts, where they feed on leaves, mate, and oviposit into leaf petioles (Schwarzlaender, 1997). Larvae hatch from eggs 7-10 days after oviposition and begin to mine down into the root crown where they feed primarily in the vascular cylinder. *Mogulones cruciger* has three instars and pupates in the surrounding soil (Schwarzlaender, 1997). In late summer, adults emerge and begin feeding on houndstongue rosettes between July and October. Oviposition begins in late August until temperatures cool and oviposition sites become unavailable. Adult weevils hibernate in leaf litter (Schwarzlaender, 1997; De Clerk-Floate and Schwarzlaender, 2002). *Mogulones cruciger* often has overlapping generations. As a consequence, eggs and larvae can be found in houndstongue roots and leaf petioles at most times of the year.

The native North American Boraginaceae species *Hackelia venusta* (Piper) St. John was chosen as a non-target species in this study because it is listed as endangered by the United States Fish and Wildlife Service (USFWS) and laboratory tests indicated that *M. cruciger* is capable of partial larval development on this species (Andreas, 2004). Its distribution is limited to one small remaining population of approximately 150 individuals

in a ponderosa pine (*Pinus ponderosa* P. & C. Lawson) and Douglas fir (*Psuedotsuga menziesii* (Mirbel) Franco) clearing in Chelan County, Washington, just south of the Canada/U.S. border (Center for Plant Conservation, 2004). Fire suppression has been an important factor in the reduction of *H. venusta* populations (Center for Plant Conservation, 2004). *Cryptantha spiculifera* and *Hackelia floribunda* were selected for this experiment because they co-occur with houndstongue at field sites in Canada (Kartesz, 1999) and were monitored in a post-release open field study (Andreas, 2004).

## Olfactometer bioassay

Our methods for an olfactometer bioassay and for electroantennogram (EAG) and GC/EAD have been adapted from those proven effective for a close relative of *M. cruciger*, the cabbage seed weevil, *Ceutorhynchus obstrictus* Marsham (*C. assimilis* Payk.) (Evans and Allen-Williams, 1992; Evans and Bergeron, 1994). The olfactometer was a four-arm configuration (Vet *et al.*, 1983) (Syntech, Hilversham, The Netherlands). We conducted preliminary 3-way choice bioassays in the olfactometer with air streams directed over intact plants of *H. floribunda*, *C. spiculifera*, houndstongue, and a blank (carrying humidified air). Intake air for all treatments was prefiltered through activated charcoal. Airflow was balanced at 300 ml/min through each arm of the olfactometer.

Illumination was with overhead fluorescent fixtures, diffused through a white, translucent plastic pail inverted over the olfactometer. The lens of a video camera was inserted through the top center of the pail to permit continuous recording of weevil behaviour during the bioassay. Twelve female weevils (having fed previously on houndstongue but not having encountered other hosts) were tested simultaneously in a single run. The weevils were placed at the center of the olfactometer and their positions and movements recorded on videotape for 30 minutes for later review and recording using a computer program (Noldus Observer, Wageningen, The Netherlands). Relative attractiveness of each source was quantified in terms of the proportion of time spent in the respective quadrants of the olfactometer.

## VOC analysis

Volatile organic compounds (VOCs) were trapped from the headspace of houndstongue, *H. venusta*, *H. floribunda*, and *C. spiculifera* with a volatile collection apparatus (Analytical Research Systems, Inc., Gainesville, FL) following methods modified from Eigenbrode *et al.* (2002). The headspace VOC profiles were compared for similarity based on the number of shared compounds detected and by calculating similarity coefficients based on occurrence and relative abundance of each component. We employed two binary coefficients, Jaccard's and Sørensen's, and one quantitative coefficient, Bray-Curtis, commonly used for ecological studies (Southwood and Henderson, 2000).

## EAG and GC/EAD/FID

EAG assay methods were modified from those of Evans and Allen-Williams (1992). Recordings were taken from single antennae on partially excised heads using sharpened stainless steel electrodes coated with an electrolyte gel. To measure antennal response to the total blend of VOCs from each species, samples of headspace volatile that had been standardized based on plant dry weight were applied in solvent to a filter paper strip and delivered to the antenna in a puff of prefiltered air. Antennal response to VOCs of each species was standardized on the basis of a response to a single concentration of linalool. Responses from ten weevils (three males and seven females) were obtained from *H. floribunda*.

Gas chromatography using EAG and flame ionization detectors (GC/EAD/FID) (Bjostad, 1998) can help identify the components of potential host VOCs that are most important for observed behavioural responses. A 1-µl sample of headspace VOCs dissolved in methylene chloride was injected onto a Shimadzu GC17-A GC (Shimadzu Corp., Kyoto Japan) fitted with a column splitter delivering approximately half the column effluent to a flame ionization detector and half via a heated transfer line to the electroantennograph (Syntech, Hilversam, The Netherlands) with the insect preparation. Temperature programme and column specifications were conducted as described in Eigenbrode *et al.* (2002). Antennal responses (expressed as mV of depolarization) were standardized based on an external sample of linalool injected through the GC/FID/EAD between each injected sample of headspace VOCs. One female was tested.

# Results

## Olfactometer bioassay

The results from the behavioural bioassay (Figure 1) indicate that *M. cruciger* females responded to host VOCs in the olfactometer. The weevils spent the smallest proportion of time in the *H. floribunda* quadrant of the olfactometer, approximately 20% of their time in *C. spiculifera* and the no-plant control quadrants, and the greatest amount of time (approximately 40 %) in the houndstongue quadrant (One-way ANOVA; F = 4.71, $P$ = 0.006).

## VOC analysis

Of the 44 VOCs detected in headspace of the four species, six were shared by all and six were unique to houndstongue. *Hackelia venusta* headspace had the most compounds in common with houndstongue, followed by *C. spiculifera* and then *H. floribunda*. All

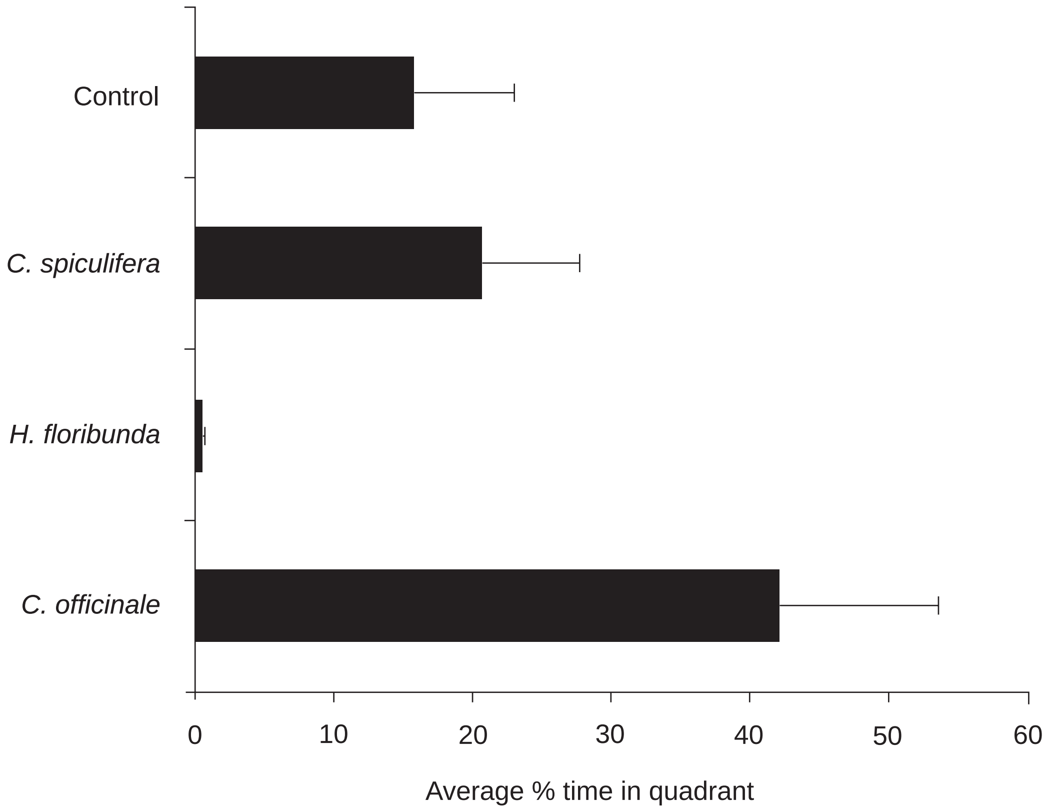


**Figure 1.** Response of *Mogulones cruciger* females to volatile organic compounds (VOCs) from houndstongue (*Cynoglossum officinale*), two non-target plant species (*Cryptantha spiculifera* and *Hackelia floribunda*) and a blank (carrying humidified air) in a four-arm olfactometer. Values are percentage of time in extremity of the test arm with stimulus ($n$=12).

three similarity coefficients follow a similar trend, with *H. venusta* most similar to houndstongue, followed by *C. spiculifera* and then *H. floribunda* (Table 1).

## EAG and GC/FID/EAD

EAG: Antennal preparations exhibited depolarization (i.e. peaks in impulses indicate excitation to particular compounds) in response to puffs of extracted headspace VOCs and this response tended to be stronger for houndstongue compared to *H. floribunda* (Figure 2) (2-way ANOVA $P$ values = 0.08, 0.16 and 0.28 for species, sex and interaction, respectively). GC/FID/EAD: The combined FID/EAD trace resulting from chromatography of *H. venusta* volatiles showed that an individual *M. cruciger* female antenna responded to a subset of the VOCs present.

# Discussion

Despite the recognized need to include host-choice behaviour and chemical ecology in host-range inves-

**Table 1.** Measures of similarity for headspace volatile profiles of *Hackelia venusta, Cryptantha spiculifera* and *Hackelia floribunda*, as compared with houndstongue (*Cynoglossum officinale*) headspace volatiles.

| Species compared to *C. officinale* | Shared components | Sørensen[a] | Jaccard[b] | Bray-Curtis[c] |
|---|---|---|---|---|
| *H. venusta* | 21 | 0.84 | 0.72 | 0.26 |
| *C. spiculifera* | 11 | 0.41 | 0.26 | 0.23 |
| *H. floribunda* | 9 | 0.36 | 0.22 | 0.22 |

[a] The Sørensen coefficient is binary in that it is based on the presence/absence of each compound. It is calculated as: $C_s = 2a/(2a + b + c)$ in which $a$ = the number of compounds held in common, $b$ = the number of compounds unique to houndstongue and $c$ = the number of compounds unique to the species compared to houndstongue. It ranges from zero for non-overlapping profiles to one for identical profiles.

[b] Jaccard similarity is also binary. It is calculated as: $C_j = a/(a + b + c)$ and also ranges from zero to one.

[c] The Bray-Curtis coefficient is a quantitative index of similarity that takes into account abundance of the components. It is calculated as $C_n = 2jN/(aN + bN)$, in which $jN$ = the sum of lesser values for those compounds shared between species and $aN$ and $bN$ are the sum of total values for each species. The coefficient scales from near one for equivalent profiles to zero.

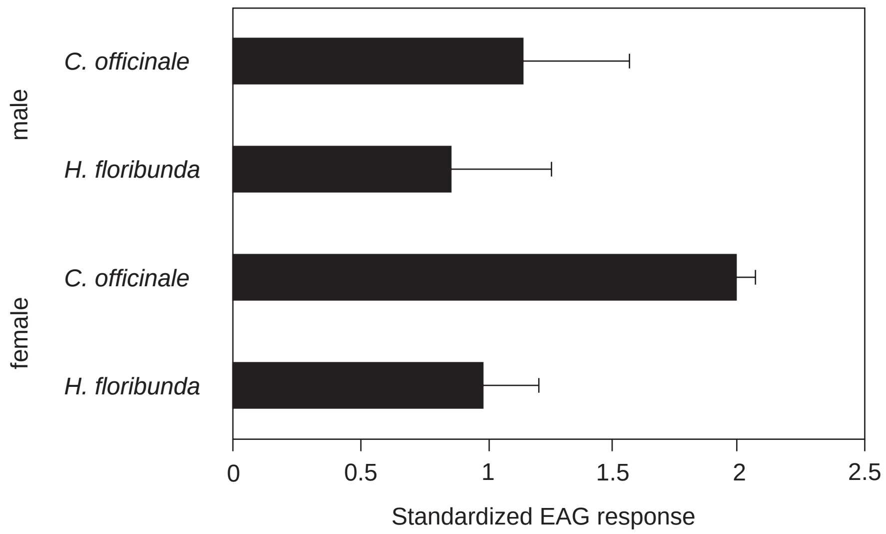


**Figure 2.** EAG responses by male and female *Mogulones cruciger* to puffs of total VOC from its host, houndstongue (*Cynoglossum officinale*) and a non-target species, *Hackelia floribunda* (response standardized relative to linalool), (n= three males and seven females).

tigations of candidate weed biocontrol agents (Briese, 2005; Sheppard *et al.*, 2005), few studies have attempted this (Heard, 2000; Hopper, 2001; Schaffner, 2001). Our data provide an illustration of the type of information that can be obtained with behavioural and chemical ecological experiments.

Our olfactometer results indicate greater responsiveness of *M. cruciger* to odour cues from houndstongue than to the tested non-target species. We did not detect directed upwind movement by the weevils in this bioassay (data not shown), indicating that *M. cruciger* dispersal among potential hosts could be undirected but that host VOCs can stimulate arrested or restricted searching behaviour on or near the host. Our bioassay has some limitations for fully understanding relevant host-selection responses to target and non-target VOCs in the field. We studied walking behaviour, but flying is likely also important in host location. The three-odour choices we offered may not represent the situation encountered from isolated non-target plants in the field. Further testing is required to address these concerns.

Our focus on VOC isolates behaviour during early stages of host selection. After contact with potential hosts, appropriate tactile and gustatory cues are required for host acceptance. Further study of the so-called ‘examination’ phase of host selection is needed for a more complete understanding of factors determining the realized host ranges of candidate biological control agents. For example, *M. cruciger* may respond to detectable pyrrolizidine alkaloids on the plant surface. Although recent work failed to find that these alkaloids could explain oviposition preference by another specialist insect, the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae) (Macel and Vrieling, 2003), the response by *M. cruciger* to these alkaloids and other gustatory cues of target and non-target plants should be examined.

The basis of apparent *M. cruciger* discrimination among odours of houndstongue and non-target species remains unknown. Some insects apparently integrate information from the VOC blend of their host plants (Roseland *et al.*, 1992), while others, including a close relative of *M. cruciger*, the cabbage seedpod weevil, *C. obstrictus*, use a few specific VOCs to orient to potential hosts (Smart and Blight, 1997). The VOC profiles of *H. floribunda*, *H. venusta* and *C. spiculifera* differ from houndstongue. *Hackelia floribunda* VOCs are the least similar to those of houndstongue. Among the three tested non-target species, the VOCs of only two, *C. spiculifera* and *H. floribunda*, were subjected to the olfactometer bioassay, where *H. floribunda* was once again less preferred. If specific compounds are required for host acceptance, they may be present in houndstongue but lacking in *H. floribunda*. Moreover, since *H. floribunda* appeared repellent in our tests, VOCs present in its profile, but lacking in houndstongue’s (i.e. benzaldehyde, undecane and dodecane) are candidate repellents. On the other hand, the weevils may integrate information from several cues during response to the VOC blends of potential hosts. For determining host-range tendencies in pre-release studies, it may not be necessary to determine these mechanisms. Our result indicates that it is feasible to include responses to cues used during host finding in assessing risks of colonization of non-targets as part of pre-release studies. Our results with *M. cruciger* indicate that *H. floribunda* may be at less risk than the other species tested here because the weevils are not attracted and possibly even

repelled by this plant, whereas *H. venusta* may be at greater risk because of the similarity of its VOC pattern to that of houndstongue.

The weevil's response to several components in the GC/FID/EAD test may help determine the basis of its host selection behaviour, as these volatiles are candidate components whose presence could attract the weevil to a non-target host that produces them. However, given that only one weevil species and one plant species was used, the results presented here simply revealed that *M. cruciger* can detect plant stimuli.

Our approach could help elucidate the risk *M. cruciger* poses to non-targets near (e.g. Andreas, 2004) and remote from colonized houndstongue infestations. In the former setting, weevils dispersing from houndstongue into the environment will be influenced by their responsiveness to VOCs and other long-distance cues from potential hosts. If there is little or no response to non-target cues (as we may have found for two non-targets), the risk of non-target attack should be reduced. Spillover effects or central excitation/sensitization potentially alter this assessment. If weevils are sufficiently abundant and mobile, regardless of responses to VOC from potential hosts, they may encounter and colonize the non-targets (spillover). If prior contact with hosts or host odours lowers acceptability thresholds to subsequently encountered plants (central excitation/sensitization), temporary non-target attack could be facilitated. Longer-distance colonization of non-targets is also potentially mediated by VOCs. Our result suggests that, while walking, weevil colonization of the two non-targets we tested are not increased due to their released VOCs.

Our approach could help assess risks of attack of other sensitive non-targets by *M. cruciger*. Three of the five endangered Boraginaceae species in the United States are within the weevil's physiological host range. Complete development was possible on *Plagiobothrys hirtus* (Greene) I.M. Johnston and *Amsinckia grandiflora* (Kleeb. ex Gray) Kleeb. ex Greene and partial development occurred on *H. venusta* (Andreas, 2004). The two remaining confamilial species that are listed as endangered in the United States, *Cryptantha crassipes* I.M. Johnston and *Plagiobothrys strictus* (Greene) I.M. Johnston were not tested. VOC profiles of these species could be tested to assess weevil attraction. *H. venusta* VOC profiles are more similar to houndstongue than others we have tested and are potentially attractive or arrestant for *M. cruciger.*

Comparisons of realized and predicted host ranges from pre-release studies (Cullen, 1990; Briese *et al.*, 1995; Clement and Cristofaro, 1995; Briese, 1999) can evaluate the soundness of prior risk-assessment procedures (Ewel *et al.*, 1999; Hopper, 2001). Selection behaviour and its phytochemical basis could, in contrast, greatly improve predictions of eventual host ranges of released agents (Marohasy, 1998; Thomas and Willis, 1998; Heard, 2000; Schaffner, 2001). By using methods similar to those presented here, behavioural and electrophysiological bioassays could form the basis of risk-assessment studies. Specifically, the release of *M. cruciger* into North America provides an opportunity to compare its realized host range with host-selection predictions based on phytochemical and behavioural studies.

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# Assessing indirect impacts of biological control agents on native biodiversity: a community-level approach

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*Summary*

The safety of biological control methods is a subject that has received considerable attention for a long time. However, apparent competition (competition due to shared natural enemies) has been neglected when considering possible impacts of biological control agents. One of the reasons for the lack of studies in this area is the difficulty in assessing and predicting indirect effects due to apparent competition. In this paper we outline a methodology to predict and measure non-target impacts of biological control agents due to apparent competition.

***Keywords***: biological control, methodology.

## Underlying rationale

Invasive species are one of the main threats to global biodiversity (Schmitz and Simberloff, 1997). Classical biological control involves the deliberate introduction of an alien species and it is viewed as a sustainable, environmentally friendly form of pest control. The safety of biological control is a subject that has received much attention, with particular concerns about the interactions between biological control agents and 'non-target' species (Pemberton and Strong, 2000; Thomas and Willis, 1998; Boettner *et al.*, 2000; Louda *et al.*, 1997). Non-target species can be affected directly, if an agent attacks a non-target host, or indirectly, for instance, when the agent shares natural enemies with native species (apparent competition, reviewed by Holt and Lawton, 1994). One of the main criteria for a certain species to be considered a safe biological control agent is its high host specificity, reducing its likelihood to directly affect native species. However, a successfully established biological control agent is an abundant resource for natural enemies present in the target ecosystem, such as parasitoids, parasites and pathogens, which can frequently be oligophagous or polyphagous (e.g. Hawkins and Goeden, 1984; Memmott *et al.*, 1994). Therefore, if these natural enemies include such an abundant food resource in their diet, their population abundance can in turn increase, creating a potential for apparent competition.

Several studies have shown that apparent competition can have strong impacts on population dynamics, either due to shared parasites (Tompkins *et al.*, 2000), predators (Muller and Godfray, 1997) or parasitoids (Morris *et al.*, 2001), as well as on community structure (e.g. herbivorous communities in Morris *et al.*, 2004, 2005; aphid-parasitoid communities in Muller and Godfray, 1999; Muller *et al.*, 1999). However, non-target impacts of an introduced biological control agent on native species through apparent competition is a subject that has not received much attention (Willis and Memmott, 2005).

If a biological control agent is effective in reducing weed abundance to low levels, then non-target impacts due to apparent competition can be minimal. However, very few pre-release studies have predicted the effectiveness of potential biological control agents in reducing target weed abundance (e.g. Buckley *et al.*, 2005; Wirfl, 2006). If an introduced agent remains at high abundance over a long period of time, the probability of non-target effects due to apparent competition is enhanced. Furthermore, non-target impacts are of particular concern for endemic species whose distribution range overlaps completely with the range of

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the weed/biological control agent, as they are the most likely species to suffer irreversible damage that may potentially lead to their extinction.

## Community level approach

Plant–insect interaction systems can be extremely complex, involving dense webs of interactions (e.g. Waser *et al.*, 1996; Memmott, 1999; Muller *et al.*, 1999; Bascompte *et al.*, 2003). Thus, to fully assess the potential indirect effects of biological control, community-level surveys are necessary. Food webs have been suggested as the appropriate way of analysing possible non-target interactions in biological control (Henneman and Memmott, 2001; Strong, 1997), since food webs enable us to ask how a biological control agent can influence native communities (Memmott *et al.*, 2007). Using food webs as predictive tools in conservation biology has, until recently, been considered an unattainable goal, as at first sight they appear very labor intensive to make and statistically difficult to analyse (Memmott *et al.*, 2007). However, community-level ecology has developed to a stage where we are capable of sampling, visualizing and analysing complex food web interactions at community-level scale (Memmott *et al.*, 2004; Dunne *et al.*, 2002; Sole *et al.*, 2001; Bersier, *et al.*, 2002; Banasek-Richter *et al.*, 2004; Cattin *et al.*, 2004).

Some studies have already used a community-level approach to look for non-target effects of biological control agents. For example, Louda *et al.*, (1997) used this approach to highlight the ability of biological control agents to disrupt communities. They demonstrated that an exotic seed-feeding biological control agent was displacing native seed feeders associated with non-target plants. Henneman and Memmott (2001) used this approach to show that in a remote area of Hawaii, 83% of parasitoids reared from native moths were biological control agents. Nowadays, this type of non-target impact (due to lack of host specificity) is avoided by using the current safety regulations governing biological control (e.g. Fowler *et al.*, 2000; Sheppard *et al.*, 2005). However, indirect non-target impacts are much harder to predict and avoid. Willis and Memmott (2005) revealed that the biological control agent, *Mesoclanis polana* (Munro) (Diptera: Tephritidae) had the potential to disrupt the native food web structure due to apparent competition, mediated by shared native parasitoids, whose population abundances exponentially increased following the population outbreak of *M. polana*. However, this study did not clearly test for impacts of the weed and the biological control agent on abundance and/or species richness of native communities. To test for such effects, repeated sampling in sites with different abundances of weed and biological control agent is needed.

In this paper we propose that food webs provide a protocol that can quantify the impact of both the alien plant and its biological control agent upon the natural community, which can then be tested using regression models.

# Suggested methodology

For a correct assessment of the impacts of the abundance of the weed and its biological control agents, two components need to be included in a post-release impact assessment programme. The first component is descriptive, involving the construction of food webs describing the patterns of trophic linkages between plants, herbivores and parasitoids in communities invaded by weeds. The second component involves statistical testing of the effects of the weed and the biological control agent abundance on native communities' abundance and species richness.

## Sampling

Selection of ten to 20 plots covering all habitats that are threatened by the weed, and covering a gradient of abundance of the weed and the biological control agent, is required. Plot size should be selected in order to include the maximum number of plant species of the field site (a suggested size of the plot is 40 · 40 m), and the plots should be at least 500 m apart, so they can be considered independent.

Ideally, all ecological niches would be studied, but it is more practical to focus on the most likely ecological niche to be affected, this being the one that includes the biological control agent in focus (e.g. seed predators, leaf miners, herbivores) and its parasitoids. Furthermore, assessing parasitism has another advantage, since it may also be highly relevant to the success and impact of the biological control programme.

Community-level sampling requires a high amount of effort. Based on a pilot project, we estimate that it will take approximately four weeks to sample 20 field sites, with two full-time people. Repeated sampling over time is needed during the seasons of higher abundances of the biological control agent to include the maximum number of species. The plots should be sampled for plants, herbivores and parasitoids monthly. The sampling and rearing methods have been described in previous literature: seed predators and their parasitoids (Memmott and Godfray, 1994); leaf herbivores and their parasitoids (Memmott *et al.*, 1994; Lewis *et al.*, 2002); and aphids and their parasitoids (Muller and Godfray, 1997; Muller *et al.*, 1999). Rearing time can vary with the biology and geographical region of the species involved. As an example, a pilot study with seed predators in Australia involved ten weeks of rearing after samples were collected.

## Determining species links

It is relatively straightforward to determine trophic links between herbivorous insects and plant species.

Determining the parasitoids of most herbivore species can be also straightforward. Immature stages of the host insect are reared in isolation until either adult hosts or parasitoids emerge (Memmott, 1999; Memmott and Godfray, 1994). Determining parasitoids of a given endophagous herbivore species (e.g. seed predator) is not as simple, as the seed predators themselves develop inside the seed. However, for many plant species there are only a few pre-dispersal seed predators and information in the literature on the food habits of the parasitoid species may be enough to identify the host. Plant–herbivore–parasitoid webs are taxonomically complex; therefore, taxonomic input is essential, although it can be time consuming and costly.

### Testing for apparent competition

Effects of the weed and biological control agent on native communities of herbivores/seed predators, parasitoids and plants can be tested by using generalized linear models (GLMs) where all possible combinations of the relevant variables (e.g. habitat, latitude, weed abundance, biological control agent abundance) will be tested. By ranking all possible models, the best model can be selected (Zuur *et al.*, 2007). If the effect of the biocontrol agent is strong enough, a significant effect will be detected over and above the effect of the weed abundance. This allows differentiating which native community patterns are significantly related to the weed abundance and/or to the biological control agent abundance. For example, if a model including the biological control agent abundance is selected as the best model (e.g. native herbivores species richness ~ habitat*biological control agent abundance), and if the contribution of the biological control agent abundance is significant to the fit of the model, we can conclude that the analysed variable is being affected by the agent.

## Conclusions

Insects form numerous key links with other species, leading to complex networks of interactions. To fully assess the post-release impacts of an introduced biological control agent, community-level studies involving quantitative data are needed. The recent practical and theoretical advances made in food-web construction and analysis allows wider applications in the field of conservation biology, such as the assessment of biological control impacts. The food-web approach suggested in this work will provide a post-release impact assessment in an understandable, applicable form for both biological control practitioners and site managers. In addition, although the methodology proposed here allows the assessment of post-release impacts, it is advisable that community-level studies are also done before the release of the biological control agents. This would reveal the native community's pre- and post-invasion 'pattern', allowing the assessment of the total magnitude of the effects of the biological control agent.

The approach presented here has recently been applied by the authors to test for indirect impacts due to apparent competition of a highly specific biological control agent, *Mesoclanis polana* Munro, recently introduced in Australia (1996) to control an invasive weed, *Chrysanthemoides monilifera* (L.) T. Norlindh, spp *rotundata* (Carvalheiro *et al*., 2008).

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# Factors affecting oviposition rate in the weevil *Rhinocyllus conicus* on non-target *Carduus* spp. in New Zealand

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*Summary*

Adults of the nodding thistle receptacle weevil, *Rhinocyllus conicus* (Froehlich) (Coleoptera: Curculionoidae), oviposit on developing thistle flower buds. Larval feeding on the receptacle prevents seed development. The weevil is known to attack several thistle species, but has clear preference for nodding thistle, *Carduus nutans* L. The effects of plant characteristics on oviposition preference and/or the size of emerging adult weevils were examined on winged and slender-winged thistles (*Carduus tenuiflorus* Curtis and *C. pycnocephalus* L., respectively). The results indicate that larger, higher seed heads on larger plants are preferred for oviposition. Larger seed heads supported the development of larger adults. This paper is part of a study looking at ecological aspects of non-target effects of thistle biological control in New Zealand. Nodding thistle flowers over an extended period of time but the two winged thistle species offer additional oviposition opportunities three to four weeks before nodding thistle flowers. The adults emerging from the winged thistle species are likely to establish a second generation, enabling this normally univoltine weevil to sustain seasonally prolonged attack on nodding thistle. Thus, proximity in space, combined with separation in time of closely related weed species, potentially enhances the performance of the oligophagous *R. conicus* as a biocontrol agent of all three thistle species.

***Keywords***: *Carduus nutans*; *C. tenuiflorus*; *C. pycnocephalus*; beneficial non-target effects.

## Introduction

*Rhinocyllus conicus* (Froehlich) (Coleoptera: Curculionoidae), nodding (musk) thistle receptacle weevil, is known to attack different thistle species but displays a clear preference for nodding thistle, *Carduus nutans* L. (Zwölfer and Harris, 1984). Eggs are laid on the developing flower buds, and the larvae burrow their way into the receptacle, where their feeding prevents seed formation (Zwölfer and Harris, 1984). The weevil, introduced to New Zealand in 1972 (Jessep, 1989), was the first agent deployed here for nodding thistle biological control.

In New Zealand, there are no native plants in the tribe Carduae, and the only crop plant belonging to the tribe, globe artichoke, is of minor economic importance (Paynter *et al.*, 2004). Thus, any thistle here — if not a weed already — could potentially become one in the future; and any impact a biological control agent such as *R. conicus* may have on any thistle here is, therefore, desirable.

We hypothesized that since *R. conicus* is highly attracted to *C. nutans* and is not likely to leave patches of *C. nutans*, then less preferred (non-target) thistle species are more likely to be attacked by the weevil in patches they share with *C. nutans*, than in patches from which *C. nutans* is absent. We selected two species that are closely related to *C. nutans* as non-target species; they were *Carduus tenuiflorus* Curtis and *C. pycnocephalus* L., and we tested the level to which they were attacked by *R. conicus* when *C. nutans* was present in close proximity, versus when it was absent. We also examined what plant characteristics may affect *R. conicus* oviposition rate among these species, in an attempt to separate this effect from that of *C. nutans* proximity.

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## Materials and methods

*Carduus pycnocephalus*, *C. tenuiflorus*, and *C. nutans* plants were grown in the University of Canterbury glasshouses from seeds collected in summer 2005 around Canterbury. In late autumn 2005 they were transplanted as small rosettes to the experimental site at the Landcare Research Lincoln campus.

At the experimental site, non-target species rosettes were organized into plots, each containing five *C. tenuiflorus* plants, five *C. pycnocephalus* plants and five *Cirsium vulgare* plants (not discussed further) in a three x five randomised arrangement, with 0.2 m between plants and 3 m between plots, for a total of 24 plots. Plots were grouped in blocks of four plots each, with at least 5 metres between blocks. Two plots in each block were randomly assigned a '*C. nutans* present' treatment, and in these, ten *C. nutans* rosettes were planted 0.2 m apart, in two rows, 0.4 m away from the non-target plants. The experiment was designed with six blocks (replicates), with four treatments per block (*C. nutans* present/absent and cage present/absent, to control for weevil density), but the cage treatment was effectively cancelled due to difficulty obtaining sufficient weevils early enough in the season, when the non-target plants were already bolting. Weevils populated the experiment independently from the surrounding environment, and thus, *R. conicus* densities were not controlled.

The longest leaf of each rosette was measured at bolting, and plant height, number of clusters, their position, and number of capitula, were recorded fortnightly. From each plot, one plant of each non-target species was randomly selected, from which all the ripe capitula were collected fortnightly and placed individually into paper bags. The height and number of capitula in the cluster were recorded for these individually collected capitula. The receptacle diameter was measured for each capitulum and any sign of attack by *R. conicus* was recorded. For each non-target species, the difference in *R. conicus* attack signs per capitulum (log transformed) were compared between treatments (*C. nutans* present vs. absent) in mixed models with Poisson distribution using R (R Development Core Team, 2006); all different plant and capitulum variables were included in the models as covariates, and non-significant variables were excluded in backwards selection using the Chi-squared test to compare between models.

Emerging *R. conicus* adults were sexed and elytra length was measured. The length was then compared between sexes and between thistle species using an F test (which included a comparison to adults that emerged from early *C. nutans* capitula in the experiment).

## Results and discussion

*Rhinocyllus conicus* attack (number of attack signs per capitulum) on the non-target species did not differ between plots with and without *C. nutans* (Table 1). *C. pycnocephalus* was attacked more than *C. tenuiflorus* (Table 1).

On both *C. tenuiflorus* and *C. pycnocephalus*, *R. conicus* preferred larger capitula on larger plants for oviposition, and attack on these species was stronger early in the spring, decreasing towards summer (equations $A_{c.t}$ and $A_{c.p}$ below).

The equations describing the effects of the measured covariates on *R. conicus* attack (number of signs per capitulum) for *C. tenuiflorus* ($A_{c.t}$) and *C. pycnocephalus* ($A_{c.p}$) are, respectively:

$$A_{c.t} = e^{(-3.6 - 1.01*\text{day} - 0.48*\text{c. height} + 0.46*\text{p. height} + 0.35*\text{diameter} + 0.09*\text{cl. size} + 0.002*\text{cap})}$$

$$A_{c.p} = e^{(-2.24 - 2.97*\text{tertiary} - 0.90*\text{secondary} + 0.52*\text{diameter} - 0.44*\text{day} + 0.29*\text{p. height} - 0.11*\text{c. height} + 0.006*\text{cap})};$$

where 'day' is day of the year on which the capitulum was collected, 'c. height' is height of the individual capitulum (mm), 'p. height' is maximum height of the plant at the time of collection (mm), 'diameter' is external diameter of the individual capitulum (mm), 'cl. size' is cluster size (number of capitula in the cluster from which the individual capitulum was collected), 'cap' is total number of capitula on the plant, 'tertiary' is for a capitulum collected from a tertiary cluster, and 'secondary' is for a capitulum collected from a secondary cluster. Similar response of *R. conicus* to plant and capitulum size was found on *C. nutans* (Sheppard *et al.*, 1994; Groenteman *et al.*, 2007; but see McNeill and Fletcher, 2005).

In New Zealand, *R. conicus* adults emerge from overwintering around November (spring), when *C. tenuiflorus* and *C. pycnocephalus* are bolting, but three to four weeks before the first *C. nutans* capitula are formed (Jessep, 1989). In those mid-spring weeks, *R. conicus* attack on the non-targets peaked at 35.7 ± 1.3% of capitula being attacked. Interestingly, even when *C. nutans* became available, oviposition on the non-targets did not cease completely, and 3.4 ± 0.8% of the

**Table 1.** Number of *Rhinocyllus conicus* attack signs per capitulum (back-transformed from log-scale means with 95% CI).

| | Thistle species | |
|---|---|---|
| Treatment | *Carduus tenuiflorus* | *Carduus pycnocephalus* |
| *Carduus nutans* present | 0.025 (0.015–0.042) | 0.11 (0.07–0.17) |
| *Carduus nutans* absent | 0.028 (0.017–0.05) | 0.10 (0.06–0.16) |

capitula still were attacked. In contrast, 100% of early *C. nutans* capitula were attacked. *Carduus tenuiflorus* and *C. pycnocephalus* capitula are considerably smaller than *C. nutans* capitula (diameters 5.66 ± 0.018 mm, 7.23 ± 0.019 mm, and 19.73 ± 0.098 mm, respectively), and they received up to 4 and 6 eggs per capitulum, respectively (with means of 0.17 ± 0.007 and 0.25 ± 0.009 for *C. tenuiflorus* and *C. pycnocephalus*, respectively). In Southern California, a biotype of *R. conicus* specialized on *C. pycnocephalus* was reported to make up to 17 (mean 1.7) penetration holes per capitulum on that plant (Goeden and Ricker, 1985). Early *C. nutans* capitula are known to receive many dozens of eggs each (Jessep, 1989; Woodburn, 1996). In accordance with oviposition levels and capitulum size, *C. tenuiflorus* and *C. pycnocephalus* were usually found to support no more than one adult per capitulum (occasionally two, and rarely three, adults per capitulum), which is comparable to the *C. pycnocephalus* specialized biotype (with mean 0.9 adults per capitulum; Goeden and Ricker, 1985), whereas *C. nutans* capitula can support the successful development of dozens of adults (R. Groenteman, personal observations). Capitulum size appeared also to affect the size of adults, with *C. tenuiflorus* producing the smallest adults, followed by *C. pycnocephalus*, and *C. nutans* with the largest individuals (elytra length 3.13 ± 0.05 mm, 3.21 ± 0.04 mm, and 3.49 ± 0.02 mm respectively, $F_{2,1058} = 38.63$, $P < 0.0001$).

Although in this experiment the non-targets *C. tenuiflorus* and *C. pycnocephalus* did not appear to be attacked more in the presence of *C. nutans*, this was probably due to the spatial scale of the experiment which, considering the distance flown by the weevils to reach the site, was negligible. Therefore, the plots could not be truly considered far enough apart to create '*C. nutans*-present and *C. nutans*-absent' treatments. Legal constraints regarding *C. nutans* propagation prevent any large scale manipulative experimentation on the species in New Zealand. *Rhinocyllus conicus* has not been considered a long distance disperser, mainly due to lack of knowledge (Sezen, 2007), and hence this was not considered a problem at the time the experiment was designed.

A field survey on a larger scale has revealed that non-targets are attacked by *R. conicus* more in the presence of *C. nutans* than in its absence (R. Groenteman, unpublished data). Furthermore, at times closer to the introduction of the weevil to New Zealand, *C. tenuiflorus* and *C. pycnocephalus* were commonly growing close to *C. nutans* (Jessep, 1989). Currently, a population of *C. nutans* in sympatry with either *C. tenuiflorus* or *C. pycnocephalus* is hard to find (R. Groenteman, personal observation). It may be that the biological control agent has, in the many years since its introduction, successfully reduced populations of the non-targets where they were adjacent to *C. nutans*. The non-targets are still abundant in New Zealand; only, not so in proximity to *C. nutans*.

To conclude, although we were unable to show that the non-target species were attacked more in the presence of the target species, we have found that *R. conicus* effectively detects patches of the non-target species, and perhaps does not leave them quickly if *C. nutans* is in close proximity. Thus, the separation in time combined with the proximity in space is possibly improving biocontrol for all of these species: The non-targets are attacked by the less-likely-to-leave weevils early in the season, thus producing a second generation of *R. conicus*. This second generation attacks *C. nutans* later in the season, after the first generation has already completed its life cycle, and when *C. nutans* is in its peak flowering in New Zealand.

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# Fortieth anniversary review of the CSIRO European Laboratory: does native range research provide good return on investment?

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*Summary*

CSIRO established its first overseas research laboratory on biological control at Montpellier in late 1966 to start a programme on skeleton weed, *Chondrilla juncea* L.). The laboratory was set up to develop the science to underpin effective biological control, by parallel studies in the native and introduced range of Australia's pests. Since establishment within a French research agency (CNRS), the facility moved in 1994 from rented facilities into a purpose-built CSIRO-owned facility, with support from Australian industry bodies and the French government. This facility has been CSIRO's largest long-term overseas investment in research. The core focus on biological control of weeds has been increasingly supplemented by other research activities that are not otherwise possible within Australia. We present an economic and scientific review of the laboratory on its 40th anniversary. The facility cost on average Aus$1.3 million (2006 $$) per year (67% on direct research activities and 33% of infrastructure and administration) and generated at least $27 benefit for Australia for every $1 invested. Staff produced 279 publications of which 159 are in journals that are currently ISI rated (average citation rate in 2007 was 14.8 per ISI journal paper).

***Keywords***: biological control, cost/benefit, foreign exploration, historical review.

## Introduction

Classical biological control aims to suppress invasive exotic pest populations by releasing specialist natural enemies, termed biocontrol agents, selected from the native range of the pest, while generating no or acceptably low non-target impacts (see Briese, 2000a). In this context, the native range of the target pest is the source of most biocontrol agents. These agents need to be found, identified, and any risks they may pose following introduction assessed by exposing them to native and commercially important species using a centrifugal phylogenetic approach (Wapshere, 1974; Briese, 2005) prior to importation into the invaded region. Countries, and their research agencies, around the world that practise classical biological control of exotic pests, tend to achieve this in one of three ways:

1. Scientific staff select potential biocontrol agents overseas during visits to the pest's native range and import them to a quarantine facility for detailed assessment. The Plant Protection Research Institute in South Africa has largely adopted this approach.
2. Contracted or collaborative research arrangements are set up with a research-provider agency to conduct the native range aspects of the research, including exploration and risk assessment. Agriculture Canada, certain US States, Landcare Research New Zealand, Queensland (Australia) and many developing countries have often adopted this approach with CABI as the dominant research provider.
   a. Research agencies set up their own overseas research facilities in the native range of the pest so that they can manage the whole biological control programme and carry out risk assess-

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ment and efficacy evaluation overseas prior to, or in conjunction with, agent importation and release. The Australian government agency, CSIRO, and USDA-ARS have largely followed this approach. Australia has or has had its own facilities in Europe, South Africa and Central America and USDA-ARS has its own facilities in Europe, South America, Asia and Australia. The USDA-ARS Australian Biological Control Research Laboratory is actually now run by CSIRO. Similarly, CSIRO also contracts survey work to the USDA South American Biological Control Laboratory.

These three options represent a progression in the scale of investment in native range research, the payoff for which has been historically argued as a) improving the chance of a successful biological control programme (Gurr and Wratten, 2000) through single-agency management of risk and efficacy assessment, release and establishment; b) greater understanding of the target through comparative ecological and genetic studies between the invaded and native range; c) greater understanding of the ecology of potential biocontrol agents and interactions with the target; and d) opportunities for enhancing the general scientific-basis of both the agent selection based on prediction of efficacy, and agent risk assessment components of biological control programmes. Additionally, the location of an agency outpost in another country offers compelling opportunities for increased international collaboration.

No previous review has evaluated any of these three options, either individually or collectively, for either a) economic return on investment, or b) scientific performance and outputs. In November 2006, the CSIRO European Laboratory (CSIRO-EL) celebrated its 40th year in Montpellier, France. This paper reviews the economic returns and scientific performance of this facility over the 40 years, including a short history of CSIRO-EL during that period.

## Research activities

Weed biological control projects undertaken at the facility in its 40 years include 30 targets across 27 genera of weeds (Table 1). Seven projects focusing on genera (*Rubus*, *Onopordum*, *Fumaria*, *Reseda*, *Sonchus*, *Vulpia* and *Convolvulus*). At least 73 species of potential agents, including 11 plant pathogens, were tested. Forty-two species of agents were released in Australia, including four plant pathogens. These were the rusts *Puccinia chondrillina* Bubak & Sydenham (four strains) for control of *Chondrilla juncea* L.; *Phragmidium violacium* (Shultz) Winter (nine strains) for *Rubus* spp.; *Uromyces heliotropii* Sred. for *Heliotropium europaeum* L.; and *Puccinia cardui-pycnocephali* Sydow for *Carduus pycnocephalus* L. and *Carduus tenuiflorus* Curtis. The chronology of these releases is given in Figure 1. Three biocontrol projects targeting insects and two targeting snails also led to releases of three insect biocontrol agents [two against *Sitona* weevil and one against Mediterranean snails, *Theba pisana* (Müller)] in Australia. Work at the facility also contributed to Australia's highly successful dung beetle project that is widely accepted to have both increased carbon and nitrogen cycling and to have lessened the public nuisance from bush flies (Edwards, 2007). Six European dung beetle species were shipped to Australia, of which *Onthophagus taurus* Schreber, *Euoniticellus fulvus* Goeze, *E. pallipes* (Fabricius), *Geotrupes spiniger* Marsham, and *Copris hispanus* L. established. Research has also been conducted, pre-emptively, against two insect pests that threaten but have yet to arrive in Australia: Russian wheat aphid, *Diuraphis noxia* (Mordvilko), and Asian gypsy moth, *Lymantria dispar* L.

## Project benefits and costs

Of the 33 classical biological control research programmes undertaken at the Montpellier facility, 11 are considered to have led to some level of success in measurable economic terms (Table 1). Most successful biological control programmes worldwide have not been subjected to economic benefit/cost analysis. Fortunately, many of the biological programmes undertaken at the facility were included in a recent economic analysis of weed classical biological control programmes across Australia (Page and Lacey, 2006). This report can be criticized for often using very limited or subjective data, but for most programmes these analyses are the best available. Certain biological control programmes had relatively in-depth economic assessments conducted prior to the report, particularly the programmes against *C. juncea* (Cullen, 1976; Marsden *et al.*, 1980), *Echium plantagineum* L. (IAC, 1985), *Carduus nutans* L. (Young and Woodburn, 2002) and *Onopordum* thistles (Meat & Livestock Australia, 1993, unpublished data). In most cases, Page and Lacey (2006) took these assessments into account, thereby ensuring more rigorous analysis and sounder conclusions. Certain biocontrol programmes based at the facility have never been economically evaluated, however, despite widespread agreement of realized benefits. These include programmes targeting *Rumex pulcher* L. and *Hypericum perforatum* L., and other programmes not targeting weeds, i.e. the dung beetle programme (Edwards, 2007).

## CSIRO European Laboratory benefit/cost analysis

Only the available benefits published in Page and Lacey (2006) could be used for a benefit assessment of the research conducted at CSIRO European Laboratory. These overall published programme benefits were

**Table 1.** Projects at CSIRO-EL between 1967 and 2007 arranged by type and chronological order, giving the years of activity, the biological control agents studied and released, and the estimated total programme (not just CSIRO-EL component) benefits and costs (from Page and Lacey, 2006) where known.

| Target pest | Dates of project at CSIRO-EL (approx.) | No. of biocontrol agents tested | No. of biocontrol agents released | Project generated a benefit? | Benefit M$ | Cost M$ |
|---|---|---|---|---|---|---|
| **Weeds biocontrol** | | | | | | |
| *Chondrilla juncea* | 1967–1999 | 4 | 4 | Yes | 1425.7 | 12.7 |
| *Echium plantagineum* | 1973–1983[a] 1987–1996 | 10 | 7 | Yes | 1201.0 | 23.0 |
| *Senecio jacobaea* | 1978–1987 | 5 | 4 | Yes | 97.2 | 3 |
| *Hypericum perforatum* | 1978–1989 | 6+1[b] | 6+1 | Yes | Nd | 3 |
| *Heliotropium europaeum* | 1978–1982 1991–1993 | 4 | 2 | No | 0 | 1.9 |
| *Rubus* spp. | 1980–1984 1999–2007 | 2 | 1[c] | Yes | 6.1 | 2.4 |
| *Rumex pulcher* | 1981–1990 | 2 | 1 | Yes | Nd | 1.3 |
| *Asphodelus fistulosus* | 1985–1990 | 1 | 0 | No | 0 | 0.2 |
| *Emex spinosa* | 1986–1990 | 1 | 1 | No | 0 | 0.2 |
| *Carduus nutans* | 1986–1995 | 5 | 3 | Yes | 81.3 | 11.9 |
| *Carduus tenuiflorus/ C. pycnocephalus* | 1986–1995 | 2 | 2 | Yes[d] | 22.5 | 1.6 |
| *Cirsium arvense* | 1986–1990 | 1 | 0 | No | 0 | 0.1 |
| *Convolvulus* spp. | 1989–1990 | 1 | 0 | No | 0 | 0.1 |
| *Onopordum* spp. | 1987–2000 | 8 | 7 | Yes | 25.0 | 7.0 |
| *Cytisus scoparius* | 1989–2002 | 8 | 3 | No | 0 | 7.0 |
| *Cirsium vulgare* | 1990–1995 | 3 | 3 | Yes | 0 | 1.5 |
| *Marrubium vulgare* | 1991–1995 | 3 | 2 | No | 0 | 1.2 |
| *Silybum marianum* | 1992–1994 | 2 | 0 | No | 0 | 0.5 |
| *Carthamus lanatus* | 1992–1999 | 1 | 0 | No | 0 | 0.4 |
| *Reseda* spp. | 1995–1999 | 1 | 0 | No | 0 | 0.2 |
| *Vulpia* spp. | 1996–1999 | 0 | 0 | No | 0 | 0.5 |
| *Lepidium* latifolium[e] | 1999–2000 | 0 | 0 | No | 0 | 0.2 |
| *Raphanus raphanistrum* | 1999–2002 | 2 | 0 | No | 0 | 0.2 |
| *Genista monspessulana* | 1999–2007 | 3 | 1[c] | No | 0 | 3.0 |
| *Fumaria* spp. | 2003–2005 | 1 | 0 | No | 0 | 0.1 |
| *Sonchus* spp. | 2003–2005 | 0 | 0 | No | 0 | 0.1 |
| *Ulex europaeus* | 2003–2007 | 0[b] | 0 | No | 0 | 0.1 |
| *Phyla canescens* | 2006– | 0 | 0 | [f] | 0 | 0. |
| *Xanthium strumarium* | ???/ | 1 | 0 | No | 0 | 0.1 |
| **Invertebrate biocontrol** | | | | | | |
| *Sitona* spp. (Lucerne weevils) | 1975–1986 | 4 | 2 | No | 0 | 1.0 |
| *Cochlicella acuta* (snail) | 1989–1994 | 3 | 1 | No | 0 | 0.3 |
| *Theba pisana* (snail) | 1989–1994 | 3 | 1 | No | 0 | 0.3 |
| *Nezara viridula*[e] (green vegetable bug) | 2006– | 0 | 0 | Evaluation research | n/a | 0.1 |
| *Maconellicoccus hirsutus*[e] (pink hibiscus mealybug) | 2000–2002 | 0 | 0 | Evaluation research | n/a | 0.1 |
| **Other biocontrol projects** | | | | | | |
| Dung[d] | 1977–1984 | 6+ | 5 | Yes | Nd | 0.6 |
| **Invertebrate risk assessment** | | | | | | |
| *Diuraphis noxia* (Russian wheat aphid) | 1989–1991 2001– | n/a | n/a | Pre-emptive research | n/a | 0.6 |
| *Lymantria dispar* [g] (Asian gypsy moth) | 1999–2004 | n/a | n/a | Pre-emptive research | n/a | 0.3 |

[a] Court injunction caused gap in research.
[b] Built on previous research in 1950s.
[c] Accidental releases.
[d] Work moved to Cordoba in Spain from 1984–1987; although Page and Lacey analyse a net benefit from this project, most of the authors dispute any real magnitude to the claimed success.
[e] U.S. target contracted research.
[f] Australian weed of S. American origin, but ecological and genetic studies are being undertaken using invasive populations in France.
[g] For Australia and New Zealand.

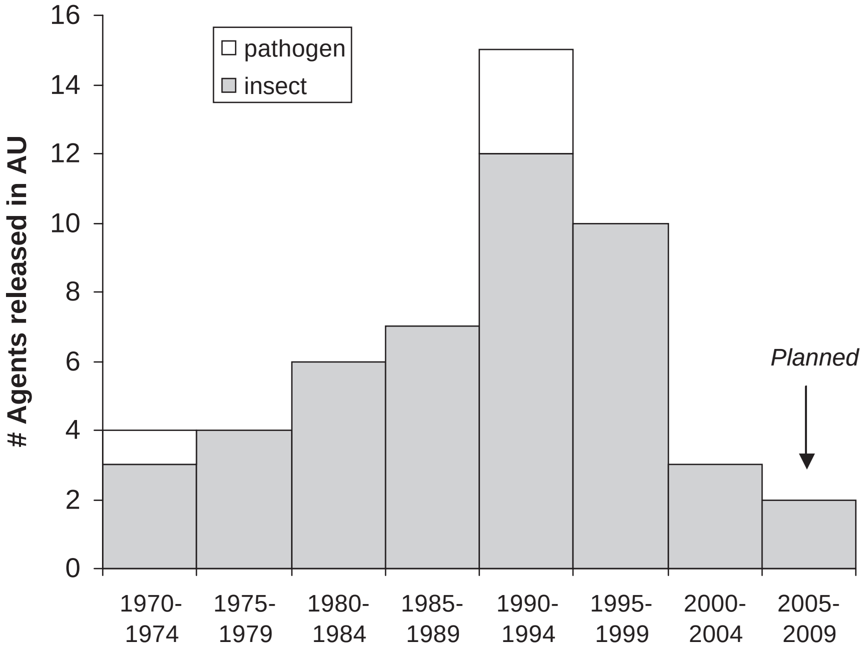


**Figure 1.** The number of weed biological control agents released into Australia from CSIRO-EL by 5-year period (data from Julien and Griffiths, 1998).

halved to reflect the contribution made by native range research at the facility; the remainder considered to result from the Australian-based activities. Estimates of the benefits to Australia from the research conducted at the facility are therefore conservative. Costs for the facility were based on estimates of the direct costs of the facility and full research project costs, rather on the project costs associated with each of the relevant programmes as used in Page and Lacey (2006).

During the 40 years, the facility has had eight officers-in-charge (OIC), 18 other scientists, 39 project officers, 7 administration staff, 14 casual staff, 34 undergraduate students, 21 postgraduate students and 17 scientific visitors (based at the facility for at least several months). Annual expenditures of the facility by CSIRO (set at 2006 Aus$ values using published historic consumer price index data) are presented in Figure 2. These expenditures from 1986–87 onwards were calculated

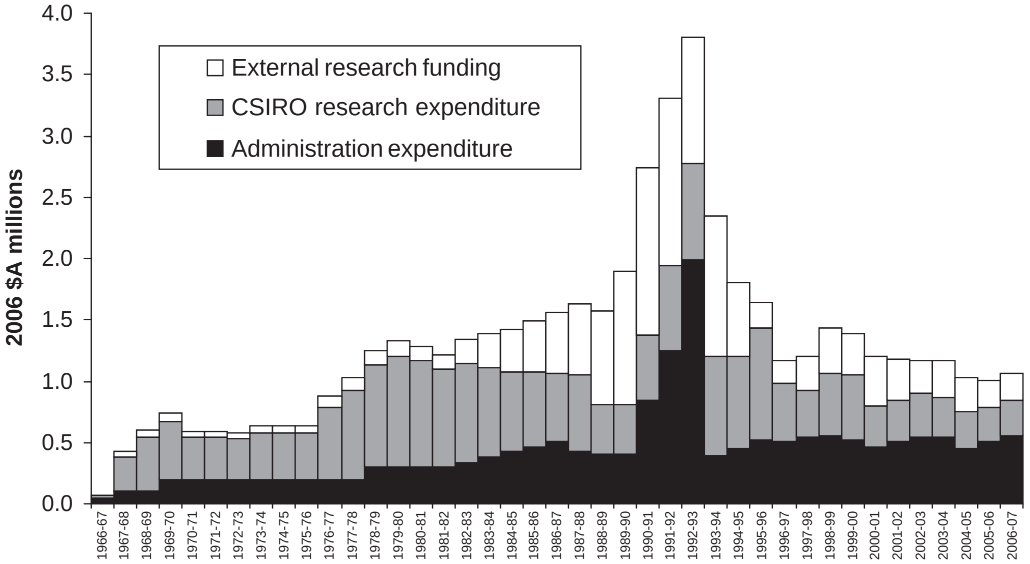


**Figure 2.** Estimated annual running costs of CSIRO-EL for a) external funding sources; b) CSIRO research project expenditure; and c) administration/buildings costs (including external support for the new laboratory constructed from 1992 to 1993) in 2006 Aus$.

based on financial records at the facility produced by the OIC. Prior to that, financial data were only available for the 1980–81 financial year. These data presented detailed and research costs and salaries per Australian and local project staff and per admin staff. These data were used to estimate costs in intervening and earlier years based on the quarterly records of the number of staff employed at the facility.

Data on external funding from primary industry research and development corporations (RDCs) were also available (Figure 2) and, where necessary, extrapolated to years for which data were not available (1966–1980 and 1983–1986); although prior to 1985, apart from some early funding from the Wheat Industry Research Council, external funding was limited. The construction of the new CSIRO European Laboratory on the Baillarguet campus between 1992 and 1993 resulted in significant additional once-off costs of c.Aus$2 million (equivalent to Aus$2.9 million in 2006 Aus$); 55% came from Australian RDC's (facility construction) and 30% from the Languedoc-Roussillon region (subsidies to servicing) and district of Montpellier (glasshouse construction).

The total conservative benefits of the research at CSIRO European laboratory are presented in Figure 3 next to the total costs of the facility over its 40-year life. The facility cost an average of Aus$1.33 million (2006 Aus$) per annum to run per year, 67% of which was spent on direct research activities and 33% on infrastructure and administrative costs. Research over the 40 years provided a Aus$1.43 billion benefit to Australian primary industries. This represents a benefit/cost ratio of 27:1, a result that is similar to the overall 23:1 benefit/cost ratio from all weed biological control research in Australia as calculated by Page and Lacey (2006). The CSIRO-European Laboratory has been an effective research investment in the native range of pests as part of Australia's overall strategy for weed biological control. In addition, it has provided the basis for successful programmes in other countries, e.g. *Sitona* weevil in New Zealand.

## Science performance

All publications from the CSIRO European Laboratory over the 40-year period were collated, including papers written by staff while at the facility and papers written based on research carried out at the facility. The list includes 279 publications of which 159 are in journals that are currently ISI rated (Figure 4). Of these, 197 were research papers that addressed agent surveys and taxonomy (11%), biology and host specificity (37%), release and evaluation (8%), agent ecology (18%) agent–target interactions (8%), target ecology, genetics and evolutionary biology (12%) and ecological theory (3%). The remainder consisted of 51 reviews, 20 technical notes and 10 book chapters. These publications were produced by 21 CSIRO staff at the facility and by

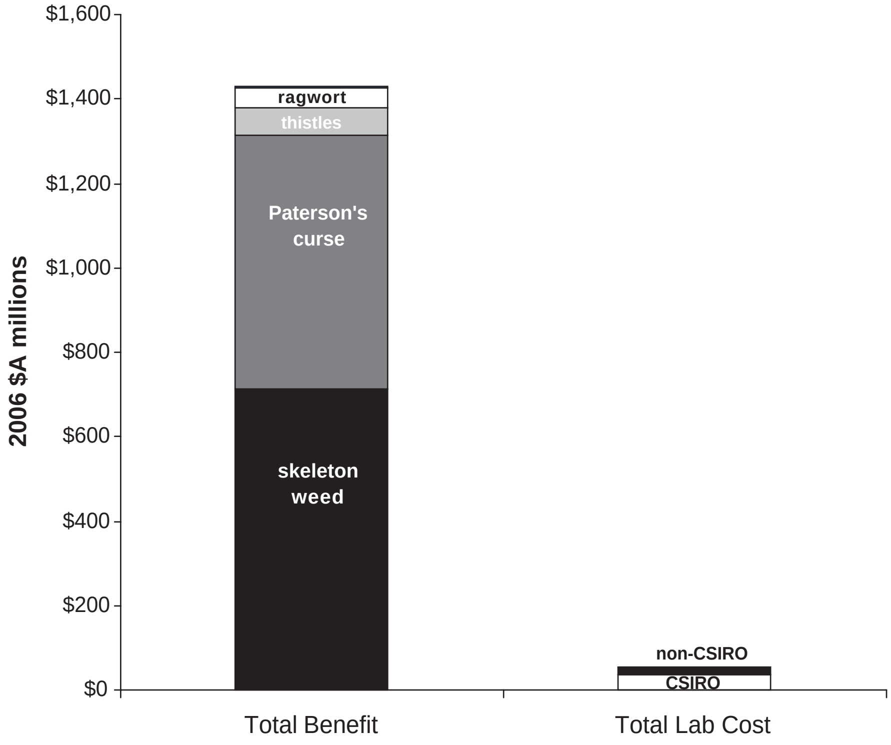


**Figure 3.** Total calculated benefits available from CSIRO-EL research in 2006 Aus$ divided by target weed and total facility costs to CSIRO, divided into direct Australian Federal Government and Primary Industry (+ French) funded components. The benefit/cost ratio is 27:1.

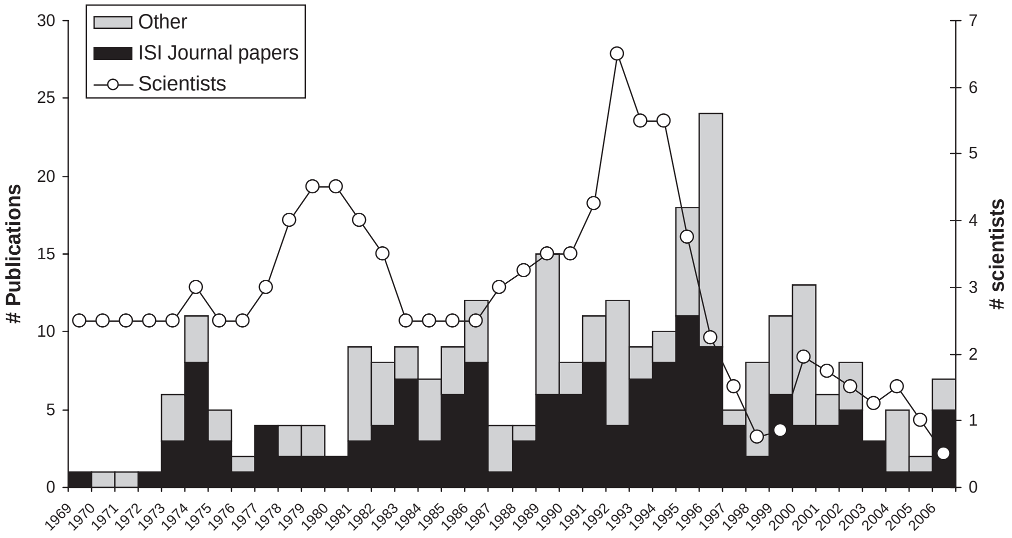


**Figure 4.** The number of publications by scientists based at CSIRO-EL or based on work predominantly done at the facility since 1966. One hundred ten scientist years (FTEs per year · 40) at the facility has generated a total of 279 publications (seven per year), 156 (four per year) in journals that are currently ISI rated.

five visitors from other agencies. Up until 2007, these papers had been cited 2,915 times on the ISI-cited reference database (since citation records began in 1985). The journal papers from journals currently ISI rated have an average citation rate of 14.8 per paper in comparison with the CSIRO-wide average of 7.9 for Agricultural Sciences and 11.6 for Ecology and Environment (CSIRO, 2007). The CSIRO-European Laboratory has also successfully produced four PhD, 11 MSc and at least 34 undergraduate project dissertations.

## Discussion

After 40 years, the CSIRO European Laboratory has had a very significant impact on the control of invasive species of European origin in Australia as well as a widely accepted significant impact to nutrient recycling by way of its contribution to activities on dung beetles (Edwards, 2007). The economic benefits achieved from research conducted at the facility have been twenty-seven times its total costs and the scientific performance of the research in terms of research publications and citations is better than the relevant average for CSIRO (CSIRO, 2007). Without similar reviews of other less costly investment models used by biological control research agencies for native range studies outlined in the Introduction, it is hard to evaluate whether the higher costs of an overseas facility yield greater net benefits or increased scientific impact. It would be hard to argue that CSIRO has had a higher success rate in biological control programmes than in countries adopting these other models, such as South Africa, Canada and New Zealand. However, CSIRO has built a strong track record and international reputation in biological control research based on the research activities at the Montpellier facility.

A benchmark paper on centrifugal phylogenetic approach to agent risk assessment (Wapshere, 1974) and his highly cited paper on global plant invasions (Lonsdale, 1999) were written while their authors were Officers-in-Charge. The facility produced the first successful programme in classical biological control using a plant pathogen (Cullen *et al.*, 1973; Cullen, 1978, Burdon *et al.*, 1981). This innovative result opened the door to using plant pathogens as biological control agents around the world (Cullen and Hasan, 1988; Barton, 2004; Morin *et al.*, 2006; Fisher *et al.*, 2007). Some of the first work on the genetic interactions between pathogen/insect and host–plant genotypes in natural systems took place at the facility (Michalakis *et al.*, 1993; Chaboudez and Burdon, 1995; Briese *et al.*, 1996; Espiau *et al.*, 1998). Work at the facility led to many key papers in characterizing insect herbivore communities (Briese *et al.*, 1994) and the population ecology of insect–plant interactions (Sheppard *et al.*, 1994; Briese, 1996, 2000b). Research over many years comparing the ecology (Paynter *et al.*, 1998; Grigulis *et al.*, 2001; Jongejans *et al.*, 2006) and evolution (O'Hanlon *et al.*, 1999) of plants in their native European range with parallel work as invaders in Australia was also carried out through the facility. Such studies are now seen as a key approach to understanding invasion and biological control processes (Hinz and Schwarzlaender, 2004; Hierro *et al.*, 2005). Collaboration between the CSIRO European Laboratory, CABI and Imperial College in the U.K. also led to an integration of ecological modelling to better understand such plant invasions (Rees and Paynter, 1997; Rees *et al.*, 1999) and their interactions with biological control

agents (Buckley *et al.*, 2005). In summary, the scientific returns to CSIRO of having a research facility in France have been substantial and have heralded many collaborative projects with USDA-ARS, CABI, French and European agencies and universities and the home agencies of visiting scientists that spent time at the facility.

## Future directions

The data presented in Figures 1, 2 and 4 show a decline in activity at the facility since a peak in the early 1990s. A number of factors contributed to this decline. First, Europe and the surrounding Old World countries are declining in importance as a source of invasive pests for Australia. Continents such as Asia, South America and Africa are the contemporary sources of many of Australia's weeds and pests. Second, the funding streams for projects that require overseas research activities are increasingly hard to attract, as governments and Rural Development Corporations seek short-term, sometimes politically motivated, measurable impacts and returns. A funding crisis throughout the 1990s for biological control research based on its inherent risk and long-term horizons has been widely recognized (Sheppard *et al.*, 2003), including by the Australian Weeds Committee, and has yet to be resolved. Third, the costs of overseas facilities have, along with the costs of scientific research generally, increased enormously, making it harder to provide sustainable project funding for a small laboratory restricted to research not possible in Australia. Increasing stringent occupational health and safety standards make increasingly expensive purpose-built research facilities a far more rational option for today's scientific needs than the rented premises used by the CSIRO Biological Control Unit for 13 years through the 1970s to 1980s. Finally, direct research collaboration between international research agencies is now the norm, through staff exchanges and the sharing of research facilities. Permanent overseas facilities are often considered too inflexible to accommodate the ever-changing international collaborative interests of research scientists. Agencies with their own overseas facilities appear increasingly isolationist to the modern scientific community. Nonetheless, the management of exotic weeds and pests and preparing for the increasing biosecurity threats associated with increased world trade ensures the maintenance of an overseas capability to undertake research on pest species before they arrive. In this latter-day context, CSIRO European Laboratory should increasingly represent a keystone to Australia's future biosecurity strategy.

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# Appendix: CSIRO European Laboratory—a potted history

In 1965 the Wheat Industry Research Council funded CSIRO to work on management options for skeleton weed, *Chondrilla juncea*. The CSIRO Entomology (CEnto) and Plant Industry (CPI) combined efforts to initiate European research activities. Doug Waterhouse (Chief CEnto) and Milton Moore (CPI), through connections with Louis Emberger (Director of the CNRS Ecology Lab in Montpellier), selected Montpellier as a climatically similar base for European studies. Tony Wapshere (CEnto) and newly appointed Richard Groves (CPI) drove to Montpellier from the Australian Embassy in Paris in late 1966 and surveyed skeleton weed populations in SW France and SE Spain.

Tony stayed in France and in November 1966 set up the CSIRO Biological Control Unit as its first Officer-in-Charge (OIC), initially employing a small team including Jeanine Lamora (later Mrs Bronner) as admin officer, using space in the CNRS Centre d'Etudes Phytosociologiques et Ecologiques. This started the first native – invasive range comparative weed ecology research (with CPI in Australia) anywhere in the world to find potential biological control agents.

By 1968 the unit of seven staff included the plant pathologist, Siraj Hasan, based at St Christol, and Louis Caresche as entomologist. After ecological studies had shown that the rust *Puccinia chondrillina* damaged infestations of *C. juncea*, Siraj's discovery of a virulent strain "IT32" at Vieste in Italy, aided by morphological matching of leaf shape (by CPI), led to the world's first successful weed biological control programme using a plant pathogen in 1971. From 1970 studies of *C. juncea* insects, mites and pathogens extended to the eastern Mediterranean centred in Thessaloniki in Greece. In 1971 the unit moved into half of a rented duplex building at 335 Ave Abbé Paul Parguel, near the experimental land and glasshouses maintained at CNRS. In 1973 the unit hosted the III International Symposium on Biological Control of Weeds (25 participants). Tony wrote his seminal paper (Wapshere, 1974) on the phylogenetic centrifugal specificity testing system and initiated preliminary surveys on several other potential weed targets, particularly *Echium plantagineum* in the western Mediterranean. In 1975 Jean-Paul Aeschlimann joined as entomologist on Hymenoptera to initiate an insect biological control programme against *Sitona* weevil. Over the next three years staff increased from 10 to 15 and Tony surveyed *C. juncea* agents in Iran. In 1978 a research outpost there involving Siraj and entomologist John Huber also initiated studies on *Heliotropium europaeum*. Tony also sent a population of the ragwort, *Senecio jacobeaea* L., flea beetle to Australia and re-initiated 1950s biocontrol work on St John's wort, *Hypericum perforatum*. In 1979 Siraj returned to the unit and Alan Kirk joined the laboratory and initiated the European arm of Australia's celebrated dung beetle programme in collaboration with Jean-Pierre Lumaret at the University of Montpellier. Alan developed an egg collection protocol and over a five-year period sent eggs of five species that successfully established in Australia.

From 1980, prospecting for plant pathogens started in earnest. *Phragmidium* rust became a candidate for blackberry, *Rubus* spp., and El Bruzzese joined the unit on secondment from the Victorian Department of Lands to work as the first State Department use of the facility. Janine Vitou joined the unit in 1981 and Isabelle Olivieri (Univ. Montpellier II) completed the unit's first French PhD thesis on thistle–pathogen interactions.

After a government review of the facility in the early 1980s, Jim Cullen took over as OIC in 1983 (on a shorter rotation cycle) and initiated biological control programmes on *Carduus* thistles while continuing work on *Hypericum*, *Senecio* and *Heliotropium*. John Scott worked in the unit from 1981–84 on *Rumex pulcher* for the Western Australia Department of Agriculture before leaving to join CSIRO. Pierre Chaboudez's electrophoresis PhD studies on *C. juncea* and *P. chondrillina* in 1985 led to work in eastern Turkey and collaboration near Ankara on a trap garden for several years. José Serin joined the unit as glasshouse manager. In 1986, Andy Sheppard joined the unit as a post doc to expand work started by Jim on the population dynamics of target weeds in the native range.

In 1987 David Briese became OIC and initiated a programme on *Onopordum* thistles. Carey Smith, from Australia, also spent two years studying the ecology of *E. plantagineum* while a court injunction prevented work in Australia. Yvette Mas became admin officer and Mireille Jourdan joined the pathology team in 1988. In 1989, Max Whitten (Chief Cento) visited and decided to support funding for a permanent facility starting a search for a suitable site. James Coupland joined the unit to initiate a programme against Mediterranean snails, *Theba pisana*, and Genevieve Martinelle joined to undertake pre-emptive research on Russian wheat aphid (RWA), *Diuraphis noxia*—both serious

pests to the Australian cereal industries. Thierry Thomann joined the unit, pushing staff numbers past 20.

During 1990–91, Jean-Paul and David worked on the new laboratory, identifying a prime 2-ha site on the proposed Agropolis Baillarguet Campus at Montferrier-sur-Lez north of Montpellier, linked to Agropolis discussions of a biocontrol campus there including French, European (never built), Australian, and American laboratories. Jim Cullen approved the site and CSIRO bought the land in 1991. Building started in mid-1992 with significant support from Australian Primary Industries and the local and regional French administration. With steady project growth, the unit reached its peak size of 25 staff with new projects on *Marrubium vulgare* L. and *Cytisus scoparius* (L.) Link. Many students from European universities undertook internships at the laboratory, providing valuable input into projects, including Yannis Michalakis who completed a PhD on *Onopordum*.

In late 1992 Richard Groves (CPI) returned as OIC and with Jean-Paul oversaw the construction and completion of the new "CSIRO European laboratory" and glasshouses. In late 1993 staff moved there, vacating glasshouses and experimental land kindly provided by CNRS since the 1960s. Collaboration with CABI on *Cytisus* started with the secondment of Quentin Paynter to the facility. On 10 October 1994, CSIRO-EL was formally opened by Barry Jones (Australian Science Minister) in the presence of Georges Frèche (Mayor of Montpellier) and Jacques Blanc (Head of Languedoc-Roussillon Region). The Institut National de la Recherche Agronomique (INRA) Centre de Biologie et de Gestion des Populations (CBGP) and the USDA-ARS-European Biological Control Laboratory (EBCL) started construction on adjacent sites in the late 1990s. Meanwhile, the INRA biocontrol and pest management team rented space in CSIRO-EL until CBGP was completed in 2002. The three laboratories associated under the "Complex Internationale de Lutte Biologique Agropolis" (CILBA), the largest group of biocontrol researchers in Europe, with shared library facilities (Le Centre Commun de Resources Documentaires) at CBGP.

In 1996 Mark Lonsdale joined as OIC and initiated studies on the ecology of annual grasses and a landmark analysis of global plant invasions (Lonsdale, 1999), and the 1st CILBA/IOBC Montpellier biocontrol conference on "Technology Transfer" was organized. Reduced project funding led to a sharp decline in staff numbers to below ten and Drs Aeschlimann and Hasan left CSIRO after long careers. A McMaster Fellowship at CSIRO-EL by Mark Rees, Imperial College London, led to a landmark invasions modelling paper (Rees *and* Paynter, 1997).

In 1998 John Scott returned as OIC. A new collaboration and joint molecular laboratory with USDA-ARS-EBCL assisted joint projects on *Raphanus raphanistrum* L., *Lepidium* spp., *Genista monspessulana* (L.) L. Johnson, and pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), and re-initiated work on *Rubus* spp. Biosecurity assessment of the Asian gypsy moth, *Lymantria dispar,* during visits by Nod Kay from New Zealand and Mamoru Matuski (CEnto), was assisted by the extensive eucalypt plantings in the grounds. In 2000 the 2nd CILBA/IOBC Montpellier biocontrol conference on "Non-target and Indirect Effects" took place and Steve Novak, Boise State University, ID, spent a sabbatical working on invasive annual grasses. The following year, the INRA team moved out and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) Entomological team and two EBCL staff moved into CSIRO-EL.

In 2002 Andy Sheppard returned as OIC and projects were initiated on *Ulex europaeus* L. and re-initiated on screening cereal varieties against worldwide populations of RWA. The 3rd CILBA/IOBC Montpellier biocontrol conference on "Genetics and Evolution" also took place. In 2006 Mic Julien became OIC and projects were initiated with a revisit by Steve Novak on lippia, *Phyla canescens* (Kunth) Greene, an invasive in Australia and France, and with EBCL, a project on the molecular evaluation of biological control agents. In 2007, the XII International Symposium on Biological Control of Weeds was held near Montpellier at La Grande Motte, organized by CSIRO-EL and USDA-EBCL (c. 250 participants) (these proceedings).

# F1 sterility: a novel approach for risk assessment of biocontrol agents in open-field trials

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Because of the growing concern of the potential risk of non-target effects, more stringent host-specificity testing is required to import and release exotic biological control agents. Appropriate host-specificity testing beyond quarantine conditions could reduce the risks of releasing biological control agents that cause negative ecological effects, and also reduce the risk that a valuable and safe biological control agent would not be approved for release. The use of reproductively inactivated insects could allow in-field host-specificity and geographical-range testing to assess the safety of exotic lepidopterans being considered as biological control agents against invasive weeds. The outstanding control of invasive cacti by *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is a classic example of successful biological control. However, *C. cactorum* became an invasive pest after its recent unintentional arrival in Florida, and currently a major effort is being developed to mitigate its negative impact. Nevertheless, the presence of *C. cactorum* in the United States and its status as both a beneficial insect and pest species provided us a unique model system to conduct proof-of-concept studies on the use of inherited (F1) sterility as a new risk management tool for assessing the safety of exotic lepidopterans being considered as biological control agents for invasive weeds.

# Impact of biocontrol agents on native biodiversity: the case of *Mesoclanis polana*

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The safety of biocontrol is a contentious issue, with particular concerns about the interactions between biocontrol agents and ‘non-target’ species. Such interactions can occur either directly, if an agent attacks a non-target host, or indirectly, when the agent affects non-target species via shared natural enemies. While there are some data on direct effects, there are very little data on indirect effects. In this talk we ask how a native food web is affected by a recently introduced biocontrol agent, *Mesoclanis polana* (Diptera: Tephritidae). While this agent will not directly affect native species (it feeds only on the target weed), it can potentially affect food-web structure indirectly via native parasitoids shared with native herbivores. Nearly 9000 seed predators were reared at the 18 field sites; 17 parasitoid species were reared from herbivores in *Chrysantomoides monilifera* seeds, with *Mesoclanis polana* the most probable host. Using a food-web approach, we ask: How does *M. polana* influence the native plant–herbivore–parasitoid community?

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# A look at host range, host specificity and non-target safety from the perspective of a plant virus as a weed-biocontrol agent

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Safety of weed biocontrol agents to non-target plants is determined primarily by means of host range/host specificity testing, often relying on the "Centrifugal-Phylogenetic" scheme to guide the selection of test plants. Recently, we have had a unique opportunity to examine the host range/host specificity of a Tobamovirus, namely Tobacco mild green mosaic tobamovirus (TMGMV), in the context of its use as a bioherbicide for *Solanum viarum*. A host range study of more than 400 plant species, subspecies or varieties in 58 plant families revealed that the virus is a pathogen adapted to plants in the Solanaceae and to a few outliers in unrelated families. The theory that host specialization follows a centrifugal-phylogenetic design appears to hold true for TMGMV at the family level. Within the family, host specificity is distinctly determined at the genus and species levels by a single gene or a few genes, with the host reaction ranging from immunity to resistance and susceptibility even within a species. The lethal hypersensitive resistance reaction seen in *S. viarum* is also different in that it is rarely observed in plant–virus interactions. Our results provide a framework to analyse the non-target risk in using TMGMV as a weed control agent.

# Novel approaches for risk assessment: feasibility studies on temporary reversible releases of biocontrol agents

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In accordance with a 1999 Executive Order adopted by the US government, federal agencies are mandated not to promote any environmental actions, e.g. biological control, unless the agencies determine that the benefits outweigh the risks and that measures will be taken to minimize potential harm. Recent case studies have shown that the risks associated with classical biological control are high because (a) host and habitat specificity are difficult to predict, and (b) natural enemy releases are normally permanent and irreversible. If the biological control agent is found not to be entirely host specific post-release, it can spread and attack non-target species in perpetuity. The potential for negative environmental impacts from biological control releases can be minimized or eliminated by adopting a precautionary approach that, in effect, makes experimental releases of candidate biological control agents temporary and reversible. The advantage of this approach is that biological control can proceed on an experimental basis before full-scale implementation. We illustrate the feasibility of this approach with two different natural enemies of Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), by proposing single sex releases of the defoliating sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae) and sterilizing the leafroller *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) with gamma radiation.

# A wolf in sheep's clothing: potential dangers of using indigenous herbivores as biocontrol agents

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Concerns about non-target effects of introduced natural enemies on native species and the existence of indigenous natural enemies attacking invasive species stimulate an interest in using indigenous herbivores for control of invasive plants. According to proponents of this strategy, using indigenous species as biocontrol agents should receive priority over introductions of foreign natural enemies. Such an approach is considered safe with low risk to native species. In contrast, we are concerned that biological control using augmentation of indigenous herbivores may lead to more serious non-target effect on native species. Indigenous natural enemies are never host specific (they have incorporated a novel host into their diet!) and often they prefer their ancestral hosts over the novel invasive ones. Even if a population or host race derived from an indigenous herbivorous insect prefers its novel invasive host, it must also be of sufficient impact to control the target invasive plant. We examined a North American indigenous herbivore, the leaf beetle *Galerucella nymphaeae,* for its potential as biological control agent of water chestnut (*Trapa natans*), in particular for its potential non-target effect on native plant species. Although speciation or host race formation often occurs in the genus *Galerucella,* the North American *G. nymphaeae* preferred its ancestral host yellow water lily (*Nuphar lutea*) over water chestnut. A competition study under different herbivory levels further indicated that mass-rearing and releasing this indigenous leaf beetle will result in increased damage to non-target native species, promoting more vigorous growth of the invasive target weed. Introduction of foreign natural enemies for biological control is not risk-free, but mass releases of indigenous herbivores may pose a more serious threat to native species than generally acknowledged.

# Impact of biological control of *Salvinia molesta* in temperate climates on biodiversity conservation

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*Salvinia molesta* is a Weed of National Significance in Australia, invading freshwater rivers and lakes and resulting in loss of biodiversity. Biological control of salvinia has been successful in tropical areas but has not yet shown an impact in temperate regions. Thus, salvinia is still considered one of the world's worst aquatic weeds and is currently spreading in many parts of the world. In Australia increased efforts have been undertaken in recent years to distribute and establish the biological control agent (*Cytrobagous salviniae*) in temperate regions. We investigated the potential long-term impact of biological control on biodiversity conservation in the Hawkesbury-Nepean River in Sydney, Australia. Differences in plant and microbe communities in salvinia infested and non-infested areas were recorded and analysed and related to water nutrient levels. The project highlights conservation priorities for revegetation and restoration to maximize species diversity following the introduction of biological control of salvinia.

# Opening Pandora's box? Surveys for attack on non-target plants in New Zealand

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The environmental safety record of weed biocontrol has recently been questioned when examples of damage to non-target plants were reported overseas. We review previous records of non-target attack and present the results of recently conducted systematic surveys to look for additional evidence of non-target damage caused by weed biological control agents that became established in New Zealand between 1929 and 2001. Our findings are discussed to determine the reliability of host-specificity testing and overall safety record of weed biological control in New Zealand and to ascertain whether lessons can be learnt that will enhance the safety of future weed biocontrol programmes.

# New biological control agents for *Cytisus scoparius* (Scotch broom) in New Zealand: dealing with the birds and the bees and predicted non-target attack to a fodder crop

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The invasive European shrub Scotch broom has detrimental impacts on farming, forestry and conservation in New Zealand. The current suite of biological control agents does not damage plants sufficiently over the entire growing season to have a major impact on broom populations and the release of additional agents: a chrysomelid leaf-feeding beetle *Gonioctena olivacea* Förster and an oecophorid stem-tying moth *Agonopterix assimilella* Treitschke was proposed. Both are narrowly oligophagous, with the potential to develop on a few closely related plant species within the tribe Genisteae. New Zealand has no native Genisteae but an exotic species, tagasaste (tree lucerne, *Cytisus proliferus* L.f. var *palmensis* H. Christ) is closely related to Scotch broom and may be affected by non-target damage. This is undesirable because tagasaste is planted to stabilize soil on marginal hill country and is a minor fodder crop in New Zealand. Furthermore, broom is valued as a pollen source by the beekeeping industry and its pods form a seasonally important food source for kererū (an endemic pigeon *Hemiphaga novaeseelandiae*). We describe a benefit/cost analysis and ecological studies performed that paved the way for the release of *G. olivacea* and *A. assimilella* in New Zealand despite objections to their use.

# Predicting risk and benefit a priori in weed biological control: a systems modelling approach

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We developed a simulation model to predict risks and benefits *a priori* for *Charidotis auroguttata,* a potential biocontrol agent for *Macfadyena unguis-cati* in Australia. Preliminary host-specificity testing of this herbivore indicated that there was limited feeding on a non-target plant, although the non-target was only able to sustain some transitions of the life cycle of the herbivore. The model incorporated herbivore, target and non-target life history, and spillover dynamics of populations of this herbivore from the target to the non-target under a variety of scenarios. Data from greenhouse and quarantine studies were used to parameterize the model and predict the relative risks and benefits of this herbivore when the target and non-target plants co-occur. Key model outputs include population dynamics on target (apparent benefit) and non-target (apparent risk) and fitness consequences to the target (actual benefit) and non-target plant (actual risk) of herbivore damage. The model predicted that non-target risk became unacceptable (i.e. significant negative effects on fitness) when the ratio of target to non-target in a given patch ranged from 1:1 to 3:2. By comparing the current known distribution of the non-target and the predicted distribution of the target we were able to identify regions in Australia where the agent may pose an unacceptable risk.

# Comparative risk assessment of *Linaria dalmatica* and *L. vulgaris* biological control

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Dalmatian (*Linaria dalmatica*) and yellow (*Linaria vulgaris*) toadflax (Scrophulariaceae) are short-lived perennial herbs of Mediterranean origin. Both species were imported to North America for horticultural purposes but have since become naturalized through multiple introductions. Toadflax diminishes floral diversity because it displaces desirable and/or native species in rangeland and forest habitats. Toadflax infestations reduce effective available grazing land because the unpalatable foliage is avoided by cattle. Herbicide treatment of toadflax is expensive due to the large acreages affected and must be repeated at impractical frequencies. Classical biological control of toadflax was initiated in the late 1960s. To date, six exotic agent species targeting the flowers, stems, foliage or roots of toadflax have established in North America. Documentation of non-target impacts in a number of classical biological control releases against other weed species suggests that a retrospective risk assessment of toadflax biological control examining ecosystem impacts of previous introductions would be well-advised at this time. Preliminary results and longer term plans to accomplish this goal will be discussed.

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# Theme 3:

# Target and Agent Selection

## Session Chair: René Sforza

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## Keynote Presenter

# Latin American weed biological control science at the crossroads

**R.W. Barreto**[1]

*Summary*

Latin America is the centre of origin of many of the invasive alien weeds threatening natural and agricultural ecosystems throughout the world. As a result, it has been an important destination for expeditions in search of natural enemies for their control. Unfortunately, the role of local scientists has been mainly that of contracted explorers, cooperating on projects aimed at exploration for classical biological control agents. This is changing as the need to confront the growing threat from alien weeds in Latin America gathers pace. Nevertheless, with limited funding and a continuing ignorance by both the general public and the decision makers about the scale of the invasive weed problem in Latin America, target selection will be critical since this will determine the long-term viability of biological control in the region. In the proactive, new role to develop biological control in Latin America, should 'easy' targets be selected, for which there has been success on other continents, or instead, should targets be more challenging, potentially confrontational, such as African grasses which threaten not only the stability of unique ecosystems but which could also have global consequences? These issues will be discussed based on experiences gained from past and present collaborative projects.

***Keywords***: target selection; agent selection; classical biological control; bioherbicides.

## Latin American weed biological control: historical background

Latin America, including the Caribbean in this paper, is the centre of origin of many of the invasive alien weed threatening systems throughout the world. For instance, 59 of the 209 worst weeds on a worldwide scale are native to Latin America (Cronk and Fuller, 1995). They include aquatic weeds such as water hyacinth, *Eichhornia crassipes* (Mart.) Solms; alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb.; capybara grass, *Hymenachne aplexicaulis* (Rudge) Nees; water lettuce, *Pistia stratiotes* L.; arrowhead, *Sagittaria motevidensis* Cham. and Schlecht.; and salvinia, *Salvinia molesta* D.S. Mitchell; and terrestrial weeds such as mistflower, *Ageratina riparia* (Regel) King and Robinson; Siam weed; *Chromolaena odorata* (L.) King and Robinson; lantana; *Lantana camara* L.; mile-a-minute *Mikania micrantha* H.B.K.; sensitive plant, *Mimosa* spp.; prickly pear, *Opuntia* spp.; strawberry guava, *Psidium cattleianum* Sabine; and Brazilian pepper tree, *Schinus terebinthifolius* Raddi.

Latin America has played a major role in weed biological control since its inception at the beginning of the 20th century. Two early pioneering projects were involved in transcontinental transfers of natural enemies aimed at *L. camara* and *Opuntia vulgaris* Miller.

### *Lantana camara*

The first explorations for natural enemies of a weed for biological control were conducted in Mexico by the Hawaii Department of Agriculture against *L. camara*. Insects were introduced into Hawaii in 1902 (Perkins and Swezey, 1924). Eight of 33 insect species that were released in Hawaii from 1902 to 1970 were established (Waterhouse and Norris, 1987). Although the accounts

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of the impact of these insects are somewhat vague, they are generally regarded as having contributed to partially controlling the weed (Goeden, 1978). *L. camara* is of worldwide importance, and interest in its biological control has been maintained to this date. In 1992, the fungus *Septoria* was introduced to combat lantana (Davis *et al.*, 1992) with excellent results (Trujillo, 2005). The case of *L. camara* is remarkable as it was the first target for biological control, and there have been around 30 projects worldwide (Broughton, 2000). The most recent introduction was a rust fungus *Prospodium tuberculatum* (Speg.) Arthur into Australia in 2001 (Ellison *et al.*, 2006). Unfortunately, no agent or combination of agents has proved sufficient to control this important weed species, and it is likely that new agents will be required. Fortunately, a highly diverse list of parasites and arthropods attack it, and new potential agents are still being found (Barreto *et al.*, 1995; Pereira and Barreto, 2000).

### *Opuntia stricta*

The control of prickly pear, *Opuntia stricta* (Haw.) Haw., in Australia, was also based on collections made in Latin America. In 1925, the moth *Cactoblastis cactorum* (Bergroth) was introduced from Argentina. In 1933, complete control was achieved over 24 million hectares of valuable land (McFadyen and Willson, 1997). This was the first example of a 'silver-bullet' effect in weed biological control, but the contribution of other arthropods and even pathogens may also have been relevant. Twelve other species of *Opuntia* spp. have been targeted by classical biological control projects using Latin American arthropods, mostly from Argentina and Mexico (Julien and Griffiths, 1998).

## The first weed biological control project targeting a weed in Latin America

The first deliberate introduction against a weed in Latin America took place in Chile in 1952 using the beetles *Chrysolina hyperici* (Forster) and *Chrysolina quadrigemina* (Suffrian) (Chrysomelidae) against St. John's wort, *Hypericum perforatum* L. This successful project (Norambuena and Ormeño, 1991) piggybacked on the successful project carried out in 1947 in the USA. Unfortunately, these introductions remained the sole examples of classical introductions into Latin America for the next 20 years.

## Pioneering work of Latin American plant pathologists in classical and inundative weed biological control

Edgardo Oehrens Bertossi, Professor of plant pathology of the Universidad Austral de Chile and often regarded as 'father of plant pathology in Chile', undertook two pioneering introductions of fungal pathogens against weeds in Latin America. The rust fungus *Phragmidium violaceum* (Schultz) Winter was introduced from Europe into Chile against blackberry, *Rubus* spp., in 1973 (Oehrens, 1977; Oehrens and Gonzales, 1974), and *Uromyces galegae* (Opiz) Sacc. was introduced, also from Europe, against goat's rue, *Galega officinalis* L. (Oehrens and Gonzales, 1974). *Phragmidium violaceum* provided effective control of *Rubus constrictus* Lefèvre and P.J. Müll., but no control resulted for *Rubus ulmifolius* Schott. (Oehrens and Gonzales, 1977; Medal, 2003). *Uromyces galegae* established but did not have any impact on goat's rue (Medal, 2003). It is interesting that these introductions were taking place almost at the same time as the rust fungus *Puccinina chondrillina* Bubak and Sydenham was being used for the first time in Australia for the biological control of skeleton weed, *Chondrilla juncea* L. (Cullen, 1974). Bertossi was ahead of his time for Latin America biological control science. He conjectured the use of rust fungi in weed biological control as early as 1963 (Oehrens, 1963), before Wilson's (1969) seminal publication, making it one of the earliest records of this kind of consideration from a plant pathologist. It is very unfortunate that Chilean pathologists have never followed Bertossi's example.

The only other account of the deliberate introduction of a pathogen as a classical biological control agent in Latin America is that of a failed attempt in Argentina to use of *P. chondrillina* as a classical biological control agent for *C. juncea* (Julien and Griffiths, 1998).

The inundative strategy involving the use of endemic fungal pathogens against invasive weeds in Latin America was explored by several research groups after being pioneered by José Tadashi Yorinori, a leading Brazilian soybean plant pathologist of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA-Soja). With collaborators, he evaluated the fungus *Bipolaris euphorbiae* (Hansford) Muchovej as a mycoherbicide against wild poinsettia, *Euphorbia heterophylla* L. (Yorinori, 1985, 1987; Yorinori and Gazziero, 1989). This work was interrupted in the 1990s due to changed research priorities in EMBRAPA-Soja and to the discovery of common biotypes of the weed that appeared to be resistant to *B. euphorbiae*. Research on this fungus as a potential mycoherbicide continues in Brazil (Marchiori *et al.*, 2001; Nechet *et al.*, 2006; Barreto and Evans, 1998).

## Continuation of searches for biological control agents by foreign scientists

Most work in Latin America continued to be limited to surveying for arthropods as potential agents for use in other continents. In the late 1950s, US Department of Agriculture (USDA)-Agricultural Research Service (ARS) scientists surveyed South America for natural enemies of *A. philoxeroides* and *E. crassipes*. Instead of short expeditions that had previously been used, USDA-

ARS chose to establish a base from which longer and more frequent surveys could be made and supported. The USDA-ARS South American Biological Control Lab (SABCL) was inaugurated in 1962 and continues its activities with excellent results until this date (Table 1). Similarly, Australian scientists from Commonwealth Scientific and Industrial Research Organisation (CSIRO) set up base in Curitiba, Brazil, later (1984) moved to Acapulco Mexico and then in 1987 to the current station in Vera Cruz, Mexico. These stations often hosted researchers from other institutions (Segura and Heard, 2004). Such strategy adopted by US and Australian scientists yielded agents that resulted in some of the most spectacular cases of success in weed biological control such as those that followed the introduction into Australia of the weevil *Cyrtobagous salviniae* Calder and Sands against *S. molesta* (Room *et al*., 1981); the introduction of *Agasicles hygrophila* Seleman and Vogt against *A. philoxeroides* into the US (Spencer and Coulson, 1976), the weevil *Neohydronomus affinis* Hustach introduced against *P. stratiotes* in Australia (Harley *et al*., 1984) and the moth *Niphograpta albiguttalis* (Warren) and the weevil *Neochetina eichhornia* Warner in the US and *Neochetina bruchi* Hustache in Australia against *E. crassipes*. Such successes were later replicated many times in different parts of the world with the same agents (e.g. Center, 1982; Julien and Griffiths, 1998).

In the last part of the 20th century, Hawaii-based entomologists such as C.J. Davis and R. Burkhart and the plant pathologist E. Trujillo introduced insects from Latin America (mainly the Caribbean) against Kosters curse, *Clidemia hirta* (L.) D. Don, as well as one fungus [*Colletotrichum gloeosporioides* (Penzig) Penzig and Sacc. (Julien and Griffiths, 1998)]. Although the fungus and a thrips *Liothrips urichi* Karny were established and Trujillo (2005) claims control levels to be adequate after repeated spraying with suspension of the fungus conidia, the weed is still a cause for concern in forest habitats (Cronk and Fuller, 1995).

Other weeds from Latin America that were targeted in Hawaiian projects were: mistflower, *Ageratina riparia* (Regel) R. King and H. Robinson, from Mexico which was spectacularly controlled with a white smut fungus *Entyloma ageratinae* Barreto and Evans (Trujillo, 2005); banana–poka, *Passiflora tarminiana* Coopens, Barney, Jørgensen and MacDugal (=*Passiflora mollissima, Passiflora tripartita*), against which insects and a fungus were released. The fungus *Septoria passiflorae* Sydenham caused significant decline of banana–poka biomass in forest areas (Trujillo, 2005).

Scientists from South Africa (Plant Protection Research Institute) have also surveyed Latin America for natural enemies of native plants that became serious weeds in South Africa. Of 31 weed species listed, 15 are from Latin America or have Latin America as part of their native range (Olckers and Hill, 1999). Some projects piggy-backed on previous studies, such as those against, *L. camara*, *E. crassipes* and *P. stratiotes*; others were initiated by South Africans. Among the recent success stories are: red water fern, *Azolla filiculoides* Lamarck, using the weevil *Stenopelmus rufinasus* Gyllenhal collected from the US, Argentina and Paraguay (Hill, 1999; Hill and Cilliers, 1999).

Intensive searches have also been made in Latin America by scientists from CAB International for biological control of pantropical weeds such as *C. odorata*, *L. camara*, *M. micrantha*, *Mimosa pigra* L. *Parthenium hysterophorus* L., and others. A recent example of work by CABI is the introduction of *Puccinia spegazzini* de Toni from Latin America to India against *M. micrantha* (Sankaran *et al*.,2008).

# Latin American weed biological control: the present

## Targeting weeds in Latin America restarted

Biological control activity restarted in Latin America in Chile (INIA-Centro Regional de Investigación Carillanca), with a programme in the 1980s against gorse, *Ulex europaeus* L., using the seed feeder *Exapion ulicis* Forster, an agent already introduced with some success from Europe into New Zealand (Norambuena *et al*., 1986; Norambuena and Piper, 2000). The gorse spider mite, *Tetranychus lintearius* Dufour, was also introduced later from Hawaii and Portugal (Norambuena *et al*., 2007).

## Interactions between foreign weed biological control scientists and Latin American scientists

Very positive actions for weed biological control science in Latin America have been the efforts by Australia-, New Zealand-, South Africa-, European- and US-based scientists to encourage active involvement of Latin American entomologists and plant pathologists in weed biological control programmes (see Table 1). Some of these involve interactions by scientists and research groups from more than two Latin American countries such as the projects on Brazilian pepper tree, *S. terebinthifolius*, and tropical soda apple, *Solanum viarum* Dunal (Gandolfo *et al*., 2007; Medal *et al*., 2002).

Many scientists from Latin America were trained in weed biological control in Europe, and US. Further, after J. Medal and D Gandolfo took part of an intensive biological control of weeds training course in Australia, they also organized a series of three courses in 2002, 2004 and 2006 in Nicaragua with attendees from numerous Latin American countries.

**Table 1.** Research groups involved with weed biological control in Latin America, their projects and status of activities.

| Organization/research leader | Status of activities | Location: city/country | Principal or recent target weeds | Approach[a] | Status of project—selected results | Selected publications | Observations |
|---|---|---|---|---|---|---|---|
| Centre for Agriculture and Biosciences International, Caribbean Latin American Station | Interrupted | Curepe/Trinidad Tobago | *Chromolaena odorata*<br>*Opuntia* spp. | ce<br>ci | Concluded<br>Concluded | Elango *et al*. (1993) | |
| Centro Agronómico Tropical de Investigación y Enseñanza | Interrupted since 1999 | Turrialba/Costa Rica | *Rottboellia cochinchinensis* (Lour.) W.D. Clayton | ci | Interrupted due political and administrative problems | Reeder and Ellison (1999), Sánchez-Garita (1999) | So far, one lost opportunity for a project with great potential for Latin America |
| Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS) and Departamento de Agronomia/Universidad Nacional del Sur—F. Anderson, R. Delhey, M. Kiehr, G. Traversa | Ongoing | Bahia Blanca/Argentina | *Cabomba caroliniana* A. Gray<br>*Nassella* spp.<br>*Phyla canescens* (Kunth) Greene | ce<br>ce<br>ce | Ongoing<br>Ongoing<br>Ongoing | Sosa *et al*. (2008) | Work with *Nassella* spp. challenged by difficulties with rust life-cycle (Anonym, 2006) |
| CSIRO Entomology Mexico Field Station—T. Heard, R. Segura | Ongoing | Veracruz/Mexico | *Mimosa pigra* L.<br>*Sida acuta* Burman f. | ce<br>ce | Concluded<br>Concluded | Ostermeyer and Grace (2007), Lonsdale *et al*. (1995), Forno *et al*. (1992). | Until now 16 agents evaluated in this lab and released against four weeds in Australia |
| Departamento de Biologia Aplicada à Agropecuária (DBAA)-Universidade Estadual Paulista Júlio de Mesquita (UNESP-Jaboticabal)—R.A. Pitelli | Ongoing | Jaboticabal/Brazil | *Eichhornia crassipes*<br>*Egeria densa* Planch.<br>*Senna obtusifolia* (L.) Irwin and Barney | in<br>in<br>in | Ongoing<br>Ongoing<br>Ongoing | Pitelli *et al.*(2007), Ávila and Pitelli (2005), Borges Neto and Pitelli (2004) | Projects mainly concentrated on endemic aquatic weeds |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Departamento de Fitopatologia (DFP)-Universidade Federal de Viçosa—R.W. Barreto | Ongoing | Viçosa/Brazil | *Commelina benghalensis* L. | in | Interrupted | See site indicated below for a complete list of publications, most representing surveys of the mycobiota of weeds in Brazil.[b] | Projects encompassing a range of native and introduced weeds in Brazil and concentrated on the study of fungal pathogens as biological control agents and also some studies on plant pathogenic nematodes. |
| | | | *Cyperus rotundus* L. | in | Ongoing | | |
| | | | *Euphorbia heterophylla* | in | Ongoing | | |
| | | | *Eichhornia crassipes* | ce | Ongoing | | |
| | | | *Hedychium coronarium* J. Koenig | ci | Ongoing | | |
| | | | *Ipomoea carnea* Jacq. | ce | Ongoing | | |
| | | | *Lantana camara* | ce | Interrupted | | |
| | | | *Macfadyena unguis-cati* (L.) Gentry | ce | Ongoing | | |
| | | | *Miconia calvescens* DC | ce | One agent introduced and established in Hawaii and Tahiti | | |
| | | | *Pereskia aculeata* Miller | ce | Ongoing | | |
| | | | *Pistia stratiotes* | ce | Finished | | |
| | | | *Psidium cattleianum* | ce | Ongoing | | |
| | | | *Saggitaria montevidensis* Cham. and Schlecht. | in | Ongoing | | |
| | | | *Schinus terebinthifolius* | ce | One agent being tested in quarantine in Florida | | |
| | | | *Tradescantia fluminensis* Vell. | ce | Ongoing | | |
| EMBRAPA-Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia (CENARGEN)—Sueli Mello | Suspended | Brasília/Brazil | *Cyperus rotundus* | in | Priorities within EMBRAPA changed to other areas | Ávila *et al*. (2005), Ávila *et al*. (2000), Borges Neto *et al*. (2000) | Scientists in the team still interested in returning to the field |
| | | | *Senna obtusifolia* | in | | | |
| EMBRAPA-Soja—J.T. Yorinori | Interrupted | Londrina/Brazil | *Euphorbia heterophylla* | in | Priorities within EMBRAPA changed to other areas | Yorinori (1985, 1987), Yorinori and Gazziero (1989) | Also problems with weed resistance to the fungus |
| Fundação Universidade Regional de Blumenau—M.D. Vitorino | Ongoing | Blumenau/Brazil | *Psidium cattleianum* | ce | Ongoing | Cuda *et al*. (2005), Hight *et al*. (2003), Vitorino *et al*. (2000). Wikler and Vitorino (2007) | Additional projects in cooperation with USDA and Plant Protection Research Institute (South Africa) |
| | | | *Schinus terebinthifolius* | ce | Ongoing | | |
| | | | *Tecoma stans* (L.) Juss. ex Kunth. | in | Ongoing | | |

**Table 1.** (Continued) Research groups involved with weed biological control in Latin America, their projects and status of activities.

| Organization/research leader | Status of activities | Location: city/country | Principal or recent target weeds | Approach[a] | Status of project—selected results | Selected publications | Observations |
|---|---|---|---|---|---|---|---|
| Instituto de Investigaciones, Agropecuaria (INIA)-Centro Regional de Investigación (CRI) Carillanca—H. Norambuena | Ongoing | Temuco/Chile | *Ulex europaeus* | ci | Ongoing | Norambuena *et al*. (2007), Norambuena and Piper (2000), Norambuena *et al*. (1986) | Almost a single-man operation—continuation at risk. |
| Instituto Mexicano de Tecnologia del Agua—M.M. Jiménez | Ongoing | Jiutepec/Mexico | *Eichhornia crassipes* and other aquatic weeds | in | Fungi and insects actually being used in the field | Jimenez and Lopez (2001), Jimenez and Charudattan (1998) | |
| Universidade Estadual do Centro-Oeste do Paraná (UNICENTRO)—C. Wikler | Ongoing | Iratí/Brazil | *Cabomba caroliniana*<br>*Psidium cattleianum*<br>*Schinus terebinthifolius*<br>*Tibouchina herbacea* (DC) Cogn. | ce<br>ce<br>ce<br>ce | Agents under evaluation in quarantine in Hawaii and Florida | Hight *et al*. (2003), Wikler *et al*. (1996) | |
| Universidad de Costa Rica—P. Hanson | Final stages | San José/Costa Rica | *Miconia calvescens* | ce | Agents under evaluation in quarantine in Hawaii | Burckhardt *et al*. (2005) | Continuation of activities unlikely after end of project. |
| Universidad Austral de Chile—E.B. Oehrens | Ended | Valdivia/Chile | *Rubus* spp.<br>*Galega officinalis* | ci<br>ci | Partial success<br>No control | Oehrens (1977), Oehrens and Gonzales (1974, 1975, 1977) | No disciples left behind after a brilliant start. |
| Universidade Federal do Paraná—H. Pedrosa-Macedo | Ongoing | Curitiba/Brazil | *Psidium cattleianum*<br>*Schinus terebinthifolius*<br>*Tradescantia fluminensis* | ce<br>ce<br>ce | Agents under evaluation in Brazil and in quarantine in Hawaii and Florida | Pedrosa-Macedo *et al*. (2007a, 2007b), Pedrosa-Macedo (2000), Medal *et al*. (1999) | Activity in this lab led to the formation of new groups (UNICENTRO and FURB) |
| USDA-ARS SABCL South American Biological Control Laboratory—J. Briano, W. Cabrera Walsh, F. McKay, C. Hernandez, A. Sosa | Ongoing | Hurlingham/Argentina | *Eichhornia crassipes*, *Alternanthera philoxeroides*, *Solanum viarum*, *Prosopis* spp., *Cabomba caroliniana* A. Gray, *Schinus terebenthifolius*, *Cardiospermum grandiflorum* Sw., *Campuloclinium macrocephalum* (Less.) DC, *Pereskia aculeata* | ce—all projects | Several successful introductions in the US and elsewhere. Several ongoing projects. | Numerous publications by a series of leading scientists such as H. Cordo, D. Gandolfo, Willie Cabrera Walsh, Cristina Fernandez, Fernando McKay, Alejandro Sosa. | This has been by far the largest and most productive team of biological control scientists in Latin America but dealing almost exclusively with arthropods |

[a] ce, Classical biological exploration of local agents for introduction outside Latin America; ci, classical biological introduction of agents against alien weeds in Latin America; in, inundative/bioherbicide.

[b] For a complete list of publications, see: http://lattes.cnpq.br/4191011304306773.

### Present status of research groups and research activities in weed biological control in Latin America

An assessment of the status of research activities in Latin America was undertaken through a search of the literature and personal contacts (Table 1). Sixteen researchers or groups have involvement with weed biological control in Latin America. Only six of 23 countries have scientists working in weed biological control: Argentina, Brazil, Chile, Costa Rica, Mexico and Trinidad Tobago. Six have been involved solely dealing with exploration for natural enemies to be used elsewhere. Three labs deal mostly with the inundative/bioherbicide approach utilizing endemic pathogens. Three labs have been involved solely with classical introductions of agents into Latin America. Unfortunately, only one of these remains active (INIA-CRI Carillanca). Additionally, four labs had activities in more than one approach. Six dealt solely with pathogens, five with arthropods and four with both. This is surprising, considering the much longer history of the use of insects in weed biological control and the great number of entomologists involved in weed biological control.

### Work at DFP/UFV (Brazil)

The Departamento de Fitopatologia, DFP/Universidade Federal de Viçosa (UFV) is one of the largest Plant Pathology departments of any university in Latin America. Weed biological control activity began there after 1994, funded by Brazilian agencies, such as Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and foreign organizations, such as the University of Hawaii and Landcare Research, New Zealand. Twelve MSc and PhD students have studied weed biological control classical (inoculative) and mycoherbicide (inundative) strategies. Four serious agricultural weeds in Brazil have been selected for mycoherbicide development; wandering jew, *Commelina benghalensis* L., purple nutsedge, *Cyperus rotundus* L., wild poinsettia, *E. heterophylla* and arrowhead, *Saggitaria montevidensis* Cham. and Schlecht. Work is advanced on the use of the fungus *Lewia chlamidosporiformans* B.S. Vieira and Barreto (Vieira and Barreto, 2005). Demonstrations of its commercial viability are presently under way.

Surveys to discover fungal pathogens attacking selected weeds in Brazil have been conducted. Recently, surveyed were: *Hedychium coronarium* J. Koenig, *Ipomoea carnea* Jacq., *L. camara*, *Macfadyena unguis-cati* (L.) Gentry, *Miconia calvescens* D.C. (Seixas *et al*., 2007), *Mitracarpus hirtus* (L.) DC (Pereira and Barreto, 2005), *Pereskia aculeata* Miller (Pereira *et al*., 2007), *P. cattleianum* (Pereira and Barreto, 2007) and *S. montevidensis* Cham. and Schlecht. Publications describe the Brazilian mycobiota of 13 plant species, and others are in preparation. These provide contributions to the field of mycology and about potential biological control agents for use in Brazil or abroad. Two of these fungi have been used: *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *Miconiae*, a pathogen of *M. calvescens* in Hawaii (Barreto *et al*., 2001) and *P. tuberculatum* in Australia for the control of *L. camara* (Ellison *et al*., 2006). A new species of *Septoria* is being evaluated for *S. terebinthifolius* in quarantine in Florida. Preliminary results of ongoing work at DFP/UFV on other weeds are presented in these proceedings (Faria *et al*., 2008; Macedo *et al*., 2008; Nechet *et al*., 2008; Pereira *et al*., 2008; Soares and Barreto, 2008; Vieira *et al*., 2008).

Other scientists of this department are becoming involved. Two nematodes were found attacking *M. calvescens*: *Ditylenchus drepanocercus* Goodey, causing angular leaf spots and a new species of *Ditylenchus* sp., which is being presently described and causes severe galling on foliage. The former nematode was studied in detail (Seixas *et al*., 2004a, 2004b), but priority is being given to the latter nematode as it is easier to manipulate and causes a more severe disease. Its evaluation has provided promising results, and it is being tested in quarantine in Hawaii. Bacteriologists were also involved after a bacterial disease was found attacking *Tradescantia fluminensis*. The etiological agent was identified as *Burkholderia andropogonis*; pathogenicity was demonstrated but host-range tests appear to discourage further evaluations of its potential for a classical introduction.

## Latin American weed biological control science at the crossroads

### The challenges of re-inaugurating classical weed biological control in Latin American countries

Latin America still holds a plethora of natural enemies of important native and exotic weeds that may be used in classical or inundative weed biological control worldwide. Sadly, the potential of the discipline for tackling weed infestations in agricultural lands and for mitigating biological invasions in Latin America remains virtually untapped. To change this, there are significant challenges to be overcome to raise the discipline's status and to maintain the structures developed by past and present researchers. Some of these issues will be discussed below.

In a recent assessment of weed biological control, for classical biological control only about 5% of nearly 1000 programmes worldwide were implemented in Latin America (Ellison and Barreto, 2004). The majority of the programmes were in the USA, Australia, South Africa, Canada and New Zealand. The paucity of programmes in Latin America was attributed to a series of factors, among which are:

1. The lack of long-term funding and tendency to withdraw funding as soon as one promising agent fails to perform well regardless of other promising agents.
2. A lack of recognition from the public, government officials and local scientists of the importance of exotic invasive weeds. Among weed scientists in Latin America, there is a persistent myth that tropical Latin America is immune to invasions by exotic plants. Ellison and Barreto (2004) refute this assumption with examples of important exotic invasions into natural, semi-natural ecosystems and in agricultural systems. Only since the early 2000s has the threat to agriculture, forestry, cattle ranching and the natural environment by introduced species (including weeds) started to be recognized in Latin America.

There is a virtual absence of examples of practical use of the inundative approach in weed biological control in Latin America. This mirrors a lack of commercial success for bioherbicides on a world scale as discussed by Evans *et al*. (2001), including reasons such as: poor target selection, poor strain selection, strain instability, mass production difficulties, low shelf-life, problems with time of application and poor formulations. Nevertheless, this has not discouraged Latin American scientists from attempting to develop such products (Table 1).

## The risk of depending on 'local heroes' and the need for a strategy for expanding and perpetuating the discipline

Of the 17 research groups listed in Table 1, five of the labs have either suspended or terminated their work in this field as a result of changed political and administrative priorities or retirement or death of the lead scientist. Further, in most of cases (except USDA-ARS SABCL and CSIRO labs), activity relies on the enthusiasm of one leading scientist. Several of these scientists are either about to retire or already retired but continuing their activities at a slowing pace. The sole example of an ongoing programme of classical weed biological control in Latin America, aimed at the weed *U. europaeus*, relies almost completely on H. Norambuena's work in INIA-CRI Carillanca, Chile. This discipline's continuity cannot rely on isolate individuals. For some labs, all the activity depends on a single or few projects, and once funding becomes scarce or the project ends, activity is likely to cease. Unfortunately, in a limited period, a drastic reduction in the number of weed biological control labs in Latin America may take place. Latin America needs urgently to have more examples such as that of J.H. Pedrosa-Macedo, a forest entomologist and weed biological control scientist that prepared a second generation of scientists that are active in the field. This depends very much on the governments and institutional recognition at international, national and regional levels of the importance of the discipline of weed biological control, allowing funding of research and the establishment of new labs.

To consolidate the discipline in Latin America, highly successful classical weed biological control programmes should be implemented as quickly as possible. Such successful programmes must receive wide publicity. Piggy-backing on other successful projects is the only way to ensure such success. Pre- and post-release ecological and economical evaluations would allow for a clear demonstration of the benefits of such projects and provide for the support of future proposals. Publicity is needed to educate the public, other scientists and the authorities, to encourage further funding and promote new scientific vocations that will guarantee a future for the activity. Even within scientific forums, there is little effort by Latin American weed biological control scientists to publicize their activities and their outstanding past record. Few Latin American weed scientists are aware of the successful history of weed biological control, the highly advantageous cost/benefit ratios demonstrated for some important programmes, or even of the fact that the majority of weed species in any country are aliens that could be targeted by classical weed biological control. A more active role should be played by the Latin American weed biological control scientists within the various discipline societies and at relevant meetings.

## New rules for collecting in Latin America: field scientists × bureaucracy

In the past, insects or fungi attacking weeds were generally regarded as irrelevant to everyone but the weed biological control scientists. Field entomologists and plant pathologists could explore distant places and collect natural enemies. This was admissible as there were no laws governing such procedures, and this remains the case for many countries. In the last two or three decades, the public and government authorities worldwide became aware of the value and the need to preserve the biodiversity of ecosystems: international agreements, such as the Convention on Biodiversity, were developed and supported by national legislations. An unfortunate consequence is that exploration for classical biological control agents is sometimes not treated separately from profit-oriented bioprospection for new drugs or other compounds. Some countries have novel anti-biopiracy legislation with highly conservative safeguards that make it difficult to conduct exploration. To collect in Latin American, indeed anywhere in the world, it is necessary to obtain updated information about the legislation concerning collecting activities for the countries to be visited. In Brazil, for example, it is mandatory to work with local collaborators and to leave duplicates of specimens in a Brazilian collection. Such cooperative links invariably prove beneficial to the programme by allowing for systematic surveys by in-country scientists and may contribute to raising a

more permanent interest for the discipline. Legal issues involved in collecting in Latin America are evolving quickly. For instance, recently in Brazil, after lobbying by the scientific community, legislation was revised, releasing all scientific collection that does not involve genes, organic molecules or extracts from native species in Brazil for commercial use from the previous bureaucratic burdens established in 2001. Fortunately, this has placed collecting biological control agents in Brazil back at the situation it was in the 1990s.

## In search of collaboration for mutual benefit

Weed biological control science in Latin America owes considerably to the weed biological control scientists of developed countries who have been actively engaged in training scientists from those countries in this field and providing encouragement, partnership and funding opportunities that allowed for several among the existing labs to start and maintain their activities. To maintain and develop these relationships so that Latin America weed biocontrol science can prosper, it is important to share resources and information and to develop training for new biological control scientists. It is important to establish cooperation on a target weed by target weed basis, as it is not fair to undertake collections of biological control agents for a wide range for weeds under a single agreement.

To increase cooperation and collaboration, there is the possibility for mutual exchange of classical biological control agents. For instance, some of the worst weeds in the Indian subcontinent are from Latin America (*E. crassipes*, *C. odorata*, *I. carnea*, *L. camara*, *M. micrantha*, *P. hysterophorus*, among others); meanwhile, among the worst weeds in Latin America are plants that are natives of India, Pakistan and neighboring countries (*C. benghalensis*, *C. rotundus*, *Dichrostachys cinerea* (L.) Wight and Arn., *H. coronarium*, *Rottboellia cochinchinensis*, *Saccharum spontaneum* L.). Brazil and Argentina are the centre of origin for some noxious weeds in South Africa (*Campuloclinium macrocephalum*, *M. unguis-cati* and *P. aculeata*), while the African grass, *Eragrostis plana* Nees, is problematic in Brazil causing severe losses to cattle ranchers (Kissmann, 1997). We should work towards developing collaborative approaches that would provide mutual benefit rather than the current mainly one-way movement of agents from Latin America.

## Target selection: a critical issue for the discipline in Latin America

The target weeds which were chosen for bioherbicide development in Latin America (Table 1) are all highly damaging in many important crops and are often intractable by chemical means thus justifying a market for a one-weed-product. In the case of classical biological control, choosing the right weed can be more difficult. An obviously target for the weed scientists may not be a priority for government or environmentalists. In Brazil, where there has been no previous history of a classical introduction against any weed, the choice of the target is a delicate issue.

There are a number of very important weeds that are also cultivated providing conflict of interest around control. Examples are *Pinus* species and fodder grasses. Clearly these are not target weeds suitable for Latin America regarding the challenge of trying to raise awareness and gain acceptance for biological control. The focus should be on one or few selected exotic weed species that will raise no conflicts and that cause significant environmental or agricultural problems so that control brings uncontroversial benefit that could be used for advertising the success of the discipline. Several weed species fit into this frame. Some were already mentioned, such as *E. plana* and *H. coronarium*, but others might be contemplated, such as *Tecoma stans* (L.) Juss. ex Kunth (Bredow *et al*., 2004). Another option is to piggy-back on a successful programme developed elsewhere in the world, for example, *Cryptostegia*, which invades extensive areas of the Brazilian northeast (Herrera and Major, 2006). A highly successful programme against this weed involving the introduction of two natural enemies from Madagascar was carried out in Australia (Tomley and Evans, 2004).

Re-opening the incomplete project against itch grass, *R. cochinchinensis* (Lour.) W.D. Clayton, may also be helpful. This project was interrupted in 1990 before the host-specific head smut fungus, *Sporisorium ophiuri* (P. Henn.) Vanky could be released (Ellison and Evans, 1995; Reeder *et al*., 1996; Reeder and Ellison, 1999; Sánchez-Garita, 1999). A renewed effort from CAB International and Centro Agronómico Tropical de Investigación y Enseñanza's (CATIE) might resolve the pending issues allow for a pioneering introduction of a weed biological control agent in Central America with potential benefits for the whole of Latin America.

The situation in Latin America is currently favorable for actions that may consolidate weed biological control and help it gain the respect as a valuable discipline that offers unique solutions to major weed problems. The moment requires firm action from the weed biological control scientists in Latin America and their cooperators.

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# Galling guilds associated with *Acacia dealbata* and factors guiding selection of potential biological control agents

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*Summary*

The Australian tree *Acacia dealbata* Link (Mimosaceae) invades natural ecosystems in both the Northern and Southern Hemispheres, including areas beyond its natural range in Australia. Biological control is under development in South Africa using the seed-feeding curculionid *Melanterius maculatus* Lea. A diverse range of galling insects occur on *A. dealbata* in Australia, most exhibiting high levels of host specificity and niche partitioning within their host. Galling insects have successfully contributed to biocontrol of other *Acacia* species in South Africa. Australian galling insects from *A. dealbata* have considerable potential for adoption as novel or complementary biocontrol agents. Factors governing the selection of potential agents are considered in the context of impact on the host, efficacy and compatibility with the utilization of the host for timber, pulp, floriculture and fire-wood harvesting, particularly in resource-poor regions of the world. The potential for biological control of *A. dealbata* in invaded habitats in Australia is also discussed.

***Keywords***: wattle, conflict of interest, agent selection.

## Introduction

Silver wattle, *Acacia dealbata* Link (Mimosaceae: Botrycephalae), is a widespread and conspicuous tree indigenous to forests and woodlands of southeastern Australia (Costermans, 1983). The species has a broad habitat range with two sub-specific taxa (subsp. *dealbata*, subsp. *subalpina*) that are delineated by altitude (Kodela and Tindale, 2001). *A. dealbata* is often abundant in early post-fire vegetation succession, where mass germination of soil-stored seed is triggered by burning. In its native habitat, *A. dealbata* provides ecosystem functions such as food and habitat for fauna (Broadhurst and Young, 2006) and fixing atmospheric nitrogen. The species' silvery bipinnate foliage and abundant production of bright yellow flowers in winter to early spring contributes to the popularity of *A. dealbata* in horticulture. Large, naturalized populations of *A. dealbata* now occur in many countries and can require management to protect natural and social assets (Sheppard *et al.*, 2006; Adair, 2008). Biological control of *A. dealbata* occurs in South Africa where large-scale invasions make other forms of suppression difficult to implement. This paper examines the role of classical biological control of *A. dealbata* using gall-forming agents and how such agents may affect commercial and utilitarian values of the host tree.

### *A. dealbata*—the invader

While *A. dealbata* is native to eastern Australia, extensive and expanding naturalized populations occur in south-west Western Australia, where the species was introduced for horticultural purposes. Although southern Western Australia has an astoundingly rich native *Acacia* flora (Hnatiuk and Maslin, 1988), there are no native Botrycephalae, and very few Western Australian acacias are large woody trees. Consequently, invasion of *A. dealbata* into the native vegetation in Western Australia may have undesirable ecological impacts, although quantitative impact data both in Australia and elsewhere are lacking. In South Africa, *A. dealbata* has been problematic as early as 1915 (Henkel, 1915) and is now a weed of national importance due to negative impacts on water management and biodiversity conservation (Le Maitre *et al.*, 2002; Nel *et al.*, 2004). More recently, in Europe, *A. dealbata* was listed as one of the

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top 20 invasive plants suggested as targets for biological control (Sheppard *et al*., 2006). Invasions in southern France post-1910 have progressively replaced local vegetation including cork oaks, l`arbousier (*Arbutus unedo* L.) and heather (http://www.worldwidewattle.com/). *A. dealbata* is also naturalized in New Zealand, western North America, Madagascar, Japan and Chile (Randall, 2002).

## Utilitarian values of *A. dealbata*

In Australia, *A. dealbata* is utilized in habitat restoration programs and urban landscaping projects. The species is not utilized commercially, although pollen used by honey bees contributes to the apiary industry. In New Zealand and North America, *A. dealbata* is utilized in horticulture but with limited economic value. In contrast, the exploitation of *A. dealbata* is well developed in southern Europe and South Africa where the species services quite different industries in each of these regions.

In Europe, *A. dealbata* was introduced around 1816 (Cavanagh, 2006) where acacias ('mimosa') are grown for horticultural and floricultural purposes. The 'mimosa' cut flower industry in France occupies around 200 ha with an estimated value of €3–4 million/year (Roland, 2006). Hybrids of *A. dealbata* and selected cultivars form the basis of the industry and produce flower crops between December and March. Whether these selections and hybrids have naturalized in Europe is uncertain, but the creation and invasion of *de novo* genotypes by hybridization can complicate classical biological control programs. Essential oils from the flowers of *A. dealbata* are used as a fixative and blending agent in the manufacture of high-grade perfumes and soaps, and the industry consumes around €1 million of refined 'mimosa' absolute per year (Roland, 2006). More recently, the French tourism industry has promoted the virtues of the 'Route de Mimosa' during the main flowering season with numerous festive activities linked to this period, undoubtedly contributing to local economies in the Bormes-les-Mimosas to Grasse region.

In South Africa, silvicultural operations use *Acacia mearnsii* De Wild. And, to a limited extent, *Acacia decurrens* Willd. *A. dealbata* is not commercially cultivated, but extensive areas of naturalized and invasive populations of *A. dealbata* in eastern South Africa are the legacy of early experimental and development programs. Resource-poor communities utilize *A. dealbata* for fuel wood, charcoal and construction timber where harvesting is carried out *ad hoc* and driven by localized domestic needs (de Neergaard *et al*., 2005). The contribution of *A. dealbata* to the regional and national economies of South Africa has not been calculated, although limited and careful extrapolation from the cost–benefit analysis undertaken for *A. mearnsii* (de Wit *et al*., 2001) could possibly be made. In the *A. mearnsii* case, biological suppression programs were strongly beneficial to the national interest.

Where *A. dealbata* threatens important assets, bona fide utilitarian values need to be taken into account when designing biological control strategies to reduce levels of conflict of interest. Historically, potential conflicts of interests are avoided by: (1) not initiating biological control programs, (2) undertaking a cost–benefit analysis and proceeding with biological control where it is in the public interest, or (3) by targeting specific organs on the host and avoiding negative impacts on utilitarian interests.

## Biological control of Australian acacias

Classical biological control of Australian acacias was pioneered in South Africa, where eight species are currently subject to active research, development or agent redistribution programs. All of these programs have succeeded in the establishment of one or more agents, and several targets are now subject to satisfactory levels of suppression (Dennill *et al.*, 1999; Hoffmann *et al*., 2002). Two general approaches to biological control of acacias have been adopted in South Africa, each largely governed by the level of conflict of interest with commercial or utilitarian interests. Economically important species (*A. mearnsii*, *Acacia melanoxylon* R. Br., *A. dealbata*, *A. decurrens* Willd.) are targeted solely for biological control of reproductive organs with seed-feeding curculionids (*Melanterius* spp.) that have no negative impacts on vegetative growth of the host plant. In contrast, acacia species of little or no economic value (*Acacia cyclops* A. Cunn. ex G. Don., *Acacia longifolia* (Andrews) Willd., *Acacia saligna* (Labill.) H.L.Wendl., *Acacia pycnantha* Benth.) are subject to biological control of a range of plant organs where *Melanterius* spp. or flower-galling Cecidomyiidae are used to target reproductive organs along with *Trichilogaster* (Hymenoptera: Pteromlaidae) or *Uromycladium* (Fungi: Uredinales), which gall vegetative organs.

In Australia, biological control of invasive acacia species that have transgressed substantial geographical barriers (trans-continental invaders) is advocated. *A. dealbata* invasions in Western Australia are suggested as targets for biological control (Adair, 2008). Biological control of *A. longifolia* in Portugal has commenced following successful control in South Africa (Sheppard *et al*., 2006).

## Galling agents and biological control strategies for Australian acacias

Galling organisms vary in their impact on the host plant depending largely on the mode of physiological interaction with the host (innate impact), gall densi-

ties, phenological synchronization, location on the host and capacity to divert and accumulate resource allocation (Hartnet and Abrahamson, 1979; Dorchin *et al.*, 2006).

Dennill (1988) outlines ecological hypotheses underpinning the successful suppression of *A. longifolia* in South Africa. In this system of 'forced commitment', diversion of host resources to gall development occurs at the expense of normal growth functions. In comparison, the flower-galling cecidomyiids *Dasineura dielsi* Rübsaamen and *Dasineura rubiformis* Kolesik proposed for biological control of Australian acacias induce gall structures with biomass and calorific allocations the same as or less than normal fruit production. Therefore, disruption to vegetative growth beyond that created by normal fruit formation is unlikely (Adair, 2005). In such cases where host trees are prevented from producing heavy fruiting loads, vegetative growth was found to be either unaffected or accelerated. This process is termed 'commitment release' (Adair, 2005) and may be applicable to situations where conflicts of interest are associated with the targeting of vegetative organs.

In the case of *A. dealbata*, a diverse assemblage of galling agents is known with a range of innate impacts.

## Galling biota of *A. dealbata* in Australia

In an extensive survey of *Acacia* in southern Australia (Adair, 2005), records and accessions of gall-forming insects collected on *A. dealbata* were extracted and combined with published data records. Thirteen gall-inducing species were recorded on *A. dealbata*: seven restricted to reproductive organs; two restricted to leaves; two restricted to stems of various size classes; one restricted to vegetative and reproductive buds; and two that attacked a range of host organs (Table 1). More than half of the taxa (61%) belonged to the Cecidomyiidae (Diptera), a family that is well-known from Australian Mimosaceae (Adair, 2005).

All recorded gall-forming biota from *A. dealbata* have restricted host ranges, at least within the Botrycephalae, with the exception of the fungus *U. notabile* Mc Alpine (Uredinales), which is recorded from numerous bipinnate species of Australian *Acacia* (Marks *et al.*, 1982), although host-specific biotypes are known to occur within this genus (Morris, 1999). *Perilampella* sp. (Pteromalidae) appears to be confined to *A. dealbata*, and ?*Cecidomyia* sp. is restricted to a small group of closely related Botrycephalae, where *A. dealbata* is its principal host. Densities of gall-forming organisms associated with *A. dealbata* are generally low, but most species are widespread within the natural distribution of this species. *Dasineura* sp. 2 appears to be restricted to south-west New South Wales.

The average dry weight of galled tissue compared to the average weight of the same un-galled organs was used in this study to indicate the general level or direction of resource partitioning to gall structures (Table 1). Galls that are heavier than normal un-galled organs (positive gall biomass ratio) indicate a possible resource sink. Conversely, galled tissues with lower biomass than the same organs without galls may indicate the absence of such a resource sink. The were more cecidogenic organisms associated with *A. dealbata* with negative to neutral gall biomass ratios (61%) than those with positive gall biomass ratios (38%; Table 1).

## Selection of potential galling agents

High costs and safety concerns in the development and release of biological control agents necessitate careful selection of organisms destined for detailed evaluation. Five selection filters are proposed here for pre-screening potential galling agents for *A. dealbata*, (1) impact efficacy, (2) host specificity, (3) conflict of interest, (4) climatic compatibility and (5) risk of parasitism.

**Impact efficacy:** Efficacy filters preceding host specificity evaluation can effectively narrow the range of organisms for further consideration and may improve the prospects for success (Raghu, *et al.*, 2006). Ecological modelling designed to identify weak points in the host's life history (Briese, 2006), together with pre-release impact assessment (McClay and Balciunas, 2006), are useful tools for quantitative efficacy evaluation. However, manipulative techniques to gauge density-based impacts of endophagous organisms, particularly on large woody plants, are somewhat problematic. While density-related impacts will be important, they remain largely untestable for large trees, except perhaps in situations where outbreak populations occur in the agent's natural range, e.g. *D. rubiformis* in Western Australia (Adair, 2005). In the case of *A. dealbata*, the innate impacts of galling organisms form the initial efficacy filter, and organisms with impact-class scores of 1 and 2 (Table 1) are dropped from further consideration. Modelling insect reproductive capacity and survivorship predictions combined with estimation of population densities likely to achieve effective damage to the host may be the only realistic way of further filtering for efficacy of *A. dealbata* agents.

**Host specificity:** Galling organisms are generally host specific (monophagous) or have a host range restricted to closely related plant species (stenophagous); (Ananthakrishnan, 1984). Nearly all galling agents on *A. dealbata* are stenophagous or polyphagous within *Acacia*. Host-specificity filtering needs to consider commercial and utilitarian interests of potentially susceptible non-target taxa and should be performed in a regional context

**Table 1.** Gall-forming organisms from *Acacia dealbata* in Australia.

| Family | Genus species | Common name | Host organ | Host range[a] | Biology[b] | Impact class[c] | Geographical distribution[d] | Conflict of interest[e] | Gall: organ biomass ratio[f] | Region excluded for biocontrol[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cecidomyiidae | *Asphondylia* sp. 1 | Seed galler | Seed | S | U, C | 3 | 2 | 1 | N | |
| | *Asphondylia* sp. 2 | Pubescent bud galler | Flower bud | S | M, C | 3 | 2 | 1 | − | A, SA,E |
| | *Asphondylia* sp. 3 | Eastern bud-seed galler | Flower bud, seed | P | M , A C | 3 | 2 | 1 | − | A, SA,E |
| | *Dasineura pilifera* | Inflated floret galler | Ovary | P | U, S | 3 | 2 | 1 | − | |
| | *Dasineura* sp. 1 | Fleshy floret galler | Ovary | S | U, S | 3 | 2 | 1 | − | |
| | *Dasineura* sp. 2 | Hollow galler | Ovary | S | ?U, S | 3 | 1 | 1 | − | A |
| | ?*Cecidomyia* sp. | Red plush galler | Ovary | S | U, C | 3 | 2 | 1 | − | |
| | Undescribed genus | Pinnule galler | Pinnule | S | ?M, S | 2 | 2 | 2 | + | A, SA, E |
| Pteromalidae | *Perilampella ?hecteaeus*[h] | Bud-shoot galler | Bud | S | U, C | 6 | 2 | 2 | + | E |
| | *Perilampella* sp.[i] | Rachis galler | Leaf rachis | M (Ad) | U, C | 5 | 3 | 2 | + | E |
| Tetrastichinae | Undetermined | Lop-sided stem galler | Stem | S | ?M, C | 5 | 3 | 3 | + | A, SA, E |
| Lepidoptera | Undetermined | Small-stem galler | Stem | S | ?M, C | 2 | 3 | 3 | N | A, SA, E |
| Uredinales | *Uromycladium notabile* | Galling rust fungus | Stem, fruit, leaf | P | M,C | 6 | 2 | 3 | + | E |

[a] Ad, *Acacia dealbata*; M, monophagous; S, stenophagous—restricted to the Botrycephalae; P, polyphagous—found on species within a number of subgenera.
[b] U, Univoltine; M, multivoltine; A, alternates between host organs; S, pupation occurs in soil; C, life cycle completed within gall.
[c] 2, Minor disruption to normal growth processes but impact unlikely to affect host fitness even in high densities; 3, impact restricted to reproductive organs and gall biomass equal to or less than fruit biomass; 5, moderate disruption to normal growth processes; 6, significant disruption to normal growth processes.
[d] 1, Restricted within natural range of *A. dealbata*; 2, widespread across natural range of *A. dealbata*; 3, distribution uncertain.
[e] 1, Low—will not conflict with commercial interests associated with Australian Acacias; 2, moderate—potential to conflict with some commercial interests; 3. high—potential to conflict with most commercial interests.
[f] −, Gall biomass is lower than host organ; N, gall biomass is approximately equal to host organ; +, gall biomass is greater than host organ.
[g] A, Australia; SA, South Africa,; E, Europe.
[h] Records from *A. mearnsii* by van den Berg (1980, unpublished records) are likely to be the result of misidentification of the host plant.
[i] Taxonomic position requires verification using molecular diagnostics, and feeding range assessed using no-choice tests.

by addressing local industry issues. Using natural host records, three regionally based host-specificity filters are established for Australia, South Africa and Europe. In Western Australia, several Botrycephalae acacias are cultivated commercially, but none are used by the silvicultural industries. Based on host specificity, all galling organisms restricted to the Botrycephalae should be considered as potential biocontrol agents for *A. dealbata*. Only *Asphondylia* sp. 3 and the polyphagous biotypes of *Uromycladium* should be excluded on this basis. In South Africa, *A. mearnsii* and *A. decurrens* are commercially exploited, and galling organisms known from these hosts that have positive gall biomass ratios and are capable of affecting vegetative growth need to be excluded. Therefore, *Asphondylia* sp. 3, a new cecidomyiid genus (pinnule galler), *Tetrastichinae* sp., the stem-galling lepidopteran, and the polyphagous biotypes of *Uromycladium* should be excluded in South Africa. In Europe, organisms that attack commercially important Botrycephalae acacias are excluded where host structures of importance are affected (Table 1).

**Conflicts of interest:** The conflict of interest filter relates to direct impacts on the targeted host, *A. dealbata*. In Europe and South Africa, organisms that directly or indirectly (e.g. due to positive gall biomass ratios) affect structures of importance are excluded. Therefore, organisms affecting vegetative organs and pre-flowering structures are excluded as potential agents for Europe. In South Africa, only organisms with potential to affect vegetative growth are excluded.

**Climatic compatibility:** Close climate matching between natural and intended areas of introduction may influence the success of biological control outcomes (Dhileepan *et al*., 2006). The galling organisms associated with *A. dealbata* occur over a broad geographic area and climate range, including considerable variation in altitude and rainfall patterns. The only clear exclusion based on climatic considerations was *Dasineura* sp. 2, where known occurrences have a low match (using Climex® and Climate®) with introduced occurrences of *A. dealbata* in Western Australia. High match levels for this species occur in Europe and South Africa (unpublished data).

**Parasitism:** Endophagous organisms tend to be susceptible to parasitism (Askew, 1980), and failure of some biocontrol programs using galling agents are attributed to high parasitism levels (Muniappan and McFadyen, 2005). Methods for predicting parasitism impacts remain elusive (Adair and Neser, 2006). However, gall-forming agents that experience low parasitism levels (<30%) have been successful in suppressing their hosts (Muniappan and McFadyen, 2005). Larger gall size and chamber number can reduce parasitism levels in some gall-forming agents (Manongi and Hoffmann, 1995), however, this association is not consistent (Waring and Price, 1989). High endemic parasitism of Australian and South African analogues of *Asphondylia* sp. 2 and *Asphondylia* sp. 3 that induce single-chambered, thin-walled galls, warrant exclusion due to the high probability of attack by parasitoids. In contrast, the galls of ?*Cecidomyia* sp. consist of long compacted hairs that surround a hard woody kernel. In Australia, ?*Cecidomyia* sp. is parasitized by a specialized dipteran (Chloropidae: *Gaurax* sp.), but remarkably few hymenopterans, which typically dominate cecidomyiid galls. The parasitism risks of this insect in a biological control context may be lower than the more simply structured *Asphondylia* spp. galls.

## Conclusions

The successful suppression of invasive Australian acacias using classical biological control has been achieved through the use of galling agents that induce a debilitating resource allocation commitment in their host (Dennill, 1988) and seed-feeding agents, either in combination or as a single-agent introduction. *A. dealbata* is utilized for commercial and domestic purposes in both the Southern and Northern Hemispheres. The biological control strategy adopted for invasive non-commercial acacias in South Africa has limited application for *A. dealbata*, except in Western Australia where the level of conflicts of interest is low. In other regions, host- and organ-specific gall-inducing organisms known from *A. dealbata* may contribute to the biological suppression of this plant. Control programs that focus on suppression of seed-producing organs to avoid conflicts of interest need to be guided by the potential of the agents to achieve ecologically meaningful levels of control. While host impacts induced by endophagous organisms creating resource sinks on vegetative growth are difficult to test or predict *a priori*, control targets for solely seed-reducing organisms are more achievable through modelling of the life history attributes and population dynamics of the host. *A. dealbata* may be a density-independent species, and therefore, suppression by seed-reducing organisms, such as *Melanterius maculatus* Lea and *Bruchophagus acaciae* (Cameron) (Hymenoptera) would need to achieve very high levels of control before population-level impacts can be obtained.

Galling organisms for *A. dealbata* are available to contribute to the reduction of reproductive output of *A. dealbata*, even in situations where the host is commercially utilized. However, a compatible combination of agents is more likely to achieve high levels of seed reduction than a single agent alone, based on the enormous resource allocation of *A. dealbata* to flower and fruit production. Seed-feeding organisms that can find food at low density levels, such as *Melanterius ventralis* Lea (Donnelly and Hoffmann, 2004), but respond rapidly to sudden increases in food availability may work well in combination with organisms that attack pre-fruiting stages of the reproductive cycle. The

sequence of introduction of combinations of biological control agents remains debatable (Impson *et al*., 2008), but introductions following a reverse phenological sequence (seed-feeders before flower-feeders) may favour the establishment of organisms that target the end of the reproductive process, which could otherwise be disadvantaged (Briese, 2006).

A series of five selection filters presented here identifies gall-inducing organisms potentially suitable for suppression of *A. dealbata* at three levels of conflict of interest: low (Australia), moderate (South Africa) and high (Europe). Efficacy of impact should precede other selection filters (Raghu *et al*., 2006), and while difficult to quantify for organisms restricted to reproductive organs on large perennial trees, manipulative techniques are technically possible (Balciunas and Burrows, 1993).

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# Biological control of *Miconia calvescens* with a suite of insect herbivores from Costa Rica and Brazil

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*Summary*

*Miconia calvescens* DC. (Melastomataceae) is an invasive tree considered the most serious threat to the natural ecosystems of Hawaii and other Pacific islands. We evaluated nine species of natural enemies that feed on inflorescences or leaves of *M. calvescens* for their potential as biological control agents, comparing their impact on the target plant, host specificity, and vulnerability to biotic interference. Among herbivores attacking reproductive structures of *M. calvescens*, a fruit-galling wasp from Brazil, *Allorhogas* sp. (Hymenoptera: Braconidae), and a flower- and fruit-feeding moth from Costa Rica, *Mompha* sp. (Lepidoptera: Momphidae), were the most promising agents studied. The sawfly *Atomacera petroa* Smith (Hymenoptera: Argidae) from Brazil was thought to have the highest potential among the defoliators evaluated.

***Keywords***: herbivory, host specificity, biotic interference.

## Introduction

*Miconia calvescens* DC. (Melastomataceae) is a small tree native to Central and South America that is considered a serious threat to natural ecosystems in Hawaii and other Pacific islands because of its ability to invade native forests (Medeiros *et al*., 1997). Its devastating effects are most evident in Tahiti, where it has displaced over 65% of the native forest and threatens many endemic species (Meyer and Florence, 1996). Herbicidal and mechanical removal are the main methods used to contain the spread of *M. calvescens*, but control is difficult and costly, especially in remote areas (Medeiros *et al*., 1997; Kaiser, 2006). Since *M. calvescens* is a tree of significant size (up to 15 m high) and prolific reproduction (Medeiros *et al*., 1997; Meyer, 1998), a combination of agents attacking vegetative and reproductive structures may be necessary to achieve effective biological control.

Several insects and pathogens have been identified as potential agents in surveys conducted in the native range of *M. calvescens* in Brazil and Costa Rica by Johnson (unpublished data) and others (Burkhart, 1995; Barreto *et al*., 2005; Picanço *et al*., 2005). In the interest of avoiding unnecessary introductions and making efficient use of limited resources to evaluate potential agents, we wish to prioritize future work and focus on highly host-specific agents that hold the greatest promise for impacting *M. calvescens* in Hawaii. Because biotic interference can be a significant obstacle to successful weed biocontrol in Hawaii, we have been attempting to predict which potential agents are most susceptible to parasitoids, predators and pathogens (Johnson, in this Proceedings). In this paper, we evaluate nine insect species that feed on inflorescences or leaves of *M. calvescens* based on impact on *M. calvescens*, host specificity and vulnerability to natural enemies.

## Methods and materials

In Brazil, we studied four species attacking *M. calvescens*: a leaf-rasping sawfly, *Atomacera petroa* Smith (Hymenoptera: Argidae); a defoliating caterpillar, *Antiblemma leucocyma* Hampson (Lepidoptera: Noctuidae); a fruit-galling wasp, *Allorhogas* sp. (Hymenoptera:

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Braconidae); and a fruit-boring weevil, *Apion* sp. (Coleoptera: Curculionoidea). In Costa Rica, we evaluated five species of Lepidoptera: an undescribed *Mompha* sp. (Momphidae), *Erora opisena* (Druce) (Lycaenidae), *Temecla paron* (Godman and Salvin) (Lycaenidae) and *Parrhasius polibetes* (Cramer) (Lycaenidae), all of which fed on inflorescences, and the leaf-roller *Ategumia lotanalis* (Crambidae).

To quantify the impact of the leaf feeders on *M. calvescens*, we observed the percentage of leaves attacked in the field, and we measured area damaged per leaf using WinFOLIA® leaf area analysis software (Regent Instruments Inc., 2003). To quantify the impact of the inflorescence feeders, the percentage of flower and fruit attacked by each species in the field were determined. Additionally, in the case of internal fruit feeding, we compared the number of seeds in infested versus healthy fruits. Host specificity of each insect was assessed through a combination of observations of plants growing in association with *M. calvescens* at field sites, laboratory feeding tests and knowledge of host ranges of related insects. Our evaluation of the potential for biotic interference was based upon evidence of attack by natural enemies in the native range and comparison with enemies known for related herbivores in Hawaii. For each insect, each criterion (impact on plant, host specificity, potential for biotic interference) was assigned a score from 1 (low) to 3 (high). An overall assessment of each potential biocontrol agent was made by summing scores across criteria.

# Results

## Impacts

Each of the fruit-feeding species caused variable levels of damage in the field, usually not attacking a high percentage of fruit but damaging moderate to high levels of seeds within the fruit they did attack, e.g. 80% and 60% reduction of seeds in attacked fruits for *Allorhogas* and *Apion* sp., respectively (Badenes-Perez and Johnson, 2007b). In addition, evidence of *Apion* sp. causing premature abscission of fruits suggests that this species could indirectly reduce viability and germination of seeds. Impacts by the three species of Lycaenidae can be very high because each larva completely consumed large portions of an inflorescence before flowering or many individual immature fruits after flowering (Badenes-Perez, unpublished data). Among defoliators, *A. leucocyma* and *A. lotanalis* were considered to have very high impact based on levels of damage seen in the field as well as leaf area consumed per insect (Badenes-Perez and Johnson, 2008). Both of these lepidopterans attacked young foliage in addition to older leaves, with potentially high costs to plant fitness. In contrast, the sawfly *A. petroa* was found to attack primarily older leaves, and each larva removed relatively modest areas of leaf tissue (Badenes-Perez and Johnson, 2007a).

## Host specificity

Gall-forming *Allorhogas* spp. represent a highly diverse group with each species being quite host specific (Hanson, personal comment), like most gall-formers tend to be (Julien and Griffiths, 1998; Dennill *et al*., 1999; Hoffmann *et al*., 2002). Specificity of *Apion* sp. has not been tested, but adults were found only on *M. calvescens* in the field. *Apion* sp. tend to be host specific and have been used with some success in weed biocontrol (Julien and Griffiths, 1998; McClay and De Clerck-Floate, 1999; Norambuena and Piper, 2000). *Mompha* sp. has been reared only from fruits of *M. calvescens* in Costa Rica, where fruits of several *Miconia* spp. and other Melastomataceae have been repeatedly sampled (Chacón, 2007). *E. opisena*, *P. polibetes* and *T. paron* were only seen on *M. calvescens* in our field surveys (Badenes-Perez, unpublished data), but no focussed host specificity studies have been conducted. Larvae of *P. polibetes* have also been found feeding on Euphorbiaceae (Zikan, 1956), Leguminoseae (D'Araujo e Silva *et al*., 1968), Malpighiaceae (http://janzen.sas.upenn.edu/) and Vochysiaceae (Diniz and Morais, 2002). Larval food plants of neotropical lycaenids are poorly known, but most are thought to be polyphagous (Downey, 1962; Robbins and Aiello, 1982). Studies in the laboratory and the native habitat of *A. leucocyma* and *A. petroa* indicated that they only attacked *M. calvescens*, while *A. lotanalis* attacked other Melastomataceae besides *M. calvescens* in the laboratory (Badenes-Perez and Johnson, 2007a; Badenes-Perez and Johnson, 2008).

## Biotic interference

*Allorhogas* sp. was sometimes attacked by a eulophid parasitoid (Hymenopetera: Eulophidae: Tetrastichinae) in its natural habitat in Brazil, but there are no *Allorhogas* sp. present in Hawaii, and it appears likely that specialized enemies of this gall wasp are absent (Badenes-Perez and Johnson, 2007b). No natural enemies of *Apion* sp. were observed in the field in Brazil, but opportunities to assess biotic interference were limited by low densities of this insect (Badenes-Perez and Johnson, 2007b). A fungal pathogen is thought to limit the effective range of biocontrol by the gorse herbivore *Apion ulicis* (Forsters) (Coleoptera: Curculionoidea) in Hawaii (Julien and Griffiths, 1998). It is therefore possible that our species from *M. calvescens* might be similarly affected. Despite the presence of several parasitoids of *Mompha* sp. in Costa Rica, rates of parasitism were relatively low (Alfaro-Alpizar, unpublished data). A relative occupying a very similar niche, *Mompha trithalama* Meyrick (Lepidoptera: Momphidae), introduced from Trinidad to Hawaii, is well established and attacks a high percentage of *Clidemia hirta* (L.) D. Don. (Melastomataceae) fruits in Hawaii (Conant, 2002), although its population dynamics and overall efficacy have not been

assessed in detail. In general, insects feeding internally in *M. calvescens* fruits are expected to escape impacts from generalist enemies in Hawaii. The probability of biotic interference in Hawaii was considered moderate to high for *E. opisena*, *P. polibetes* and *T. paron* because, feeding externally, they would be exposed to a variety of generalist enemies of Lepidoptera. Although they have not been well studied, there are a few species of native and introduced lycaenids in Hawaii that may already have specialized enemies. The mimetic appearance of our three species on *M. calvescens* may, however, help them avoid some predators and parasitoids. In fact, parasitism of these species in their native Costa Rican range was low (Badenes-Perez and Johnson, 2007a).

Biotic interference was considered highly probable for *A. leucocyma* because of the high levels of parasitism found in Brazil (Badenes-Perez and Johnson, 2008) and because of high parasitism of *Antiblemma acclinalis* Hübner (Lepidoptera: Noctuidae), established but apparently ineffective as a biological control agent of *C. hirta* in Hawaii (Conant, 2002). Biotic interference also seems likely for *A. lotanalis* because several species of parasitoids and a hemipteran predator were observed attacking it in Costa Rica (Castillo-Castillo, unpublished data) and because another biocontrol agent of *C. hirta*, *Ategumia matutinalis* (Guenee) (Lepidoptera: Crambidae), also appears to be highly parasitized in Hawaii (Conant, 2002). In contrast to these lepidopterans, the sawfly *A. petroa* was considered less likely to be attacked in Hawaii because no parasitoids and predators were observed in the natural habitat of *A. petroa* in Brazil and because there are no native species of Argidae and only two other introduced sawflies in Hawaii (Badenes-Perez and Johnson, 2007a).

When the criteria of impact, specificity and biotic interference were considered together, *Allorhogas* sp. and *Mompha* sp. emerged as the strongest candidates among herbivores attacking reproductive structures of *M. calvescens* (Table 1). Other insects showing relatively high overall potential for biological control were the defoliator *A. petroa* and the inflorescence feeder *Apion* sp. Less likely to become effective biological control agents of *M. calvescens* were *A. leucocyma*, *A. lotanalis*, *E. opisena*, *T. paron* and *P. polibetes* because of their high probability of experiencing biotic interference in Hawaii and/or the possibility of low host specificity.

## Discussion

Our assessment of the potential of the insects studied as biological control agents of *M. calvescens* may be preliminary but is still helpful as an initial evaluation. Other insects being evaluated as biocontrol agents of *M. calvescens* that have not been included here are: the fruit-feeding weevil *Anthonomus monostigma* Champion (Coleoptera: Curculionidae) (Chacón, 2007), the stem/leaf-feeding weevil *Cryptorhynchus melastomae* Champion (Coleoptera: Curculionidae) (Reichert, 2007), the sap-sucking *Diclidophlebia* spp. (Hemiptera: Psyllidae) (Morais, 2007; Burckhardt *et al.*, 2005), and the defoliating caterpillar *Euselasia chrysippe* Bates (Lepidoptera: Riodinidae) (Allen, 2007). As additional information becomes available, insects will need to be re-evaluated. Other practical factors that affect prioritization are the viability of insect rearing and the difficulty to obtain permits to export insects from native areas.

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**Table 1.** Nine insect species selected as potential biological control agents of *Miconia calvescens* based on impact, host specificity and probability of biotic interference in Hawaii. For each insect, each criterion was assigned a score from 1 to 3 indicated in parenthesis: 1 = low, 2 = moderate or 3 = high. Risks of biotic interference were assigned negative scores. Finally, an overall assessment of each potential biocontrol agent was made by summing scores across criteria.

| Insect species | Plant part attacked | Impact on plant | Host specificity | Probability of biotic interference in Hawaii | Overall potential for biological control |
|---|---|---|---|---|---|
| *Allorhogas* sp. | Inflorescences | High (3) | High (3) | Low[b] (−1) | High (5) |
| *Mompha* sp. | Inflorescences | High (3) | High (3) | Low[b,c] (−1) | High (5) |
| *Apion* sp. | Inflorescences | High (3) | Unknown[a] (3) | Moderate[b,c] (−2) | Moderate-High (4) |
| *Erora opisena* | Inflorescences | High (3) | Unknown[a] (2) | Moderate[b] (−2) | Low-Moderate (3) |
| *Temecla paron* | Inflorescences | High (3) | Unknown[a] (2) | Moderate[b] (−2) | Low-Moderate (3) |
| *Parrhasius polibetes* | Inflorescences | High (3) | Low (1) | Moderate[b] (−2) | Low (2) |
| *Atomacera petroa* | Leaves | Moderate (2) | High (3) | Low[a] (−1) | Moderate-High (4) |
| *Antiblemma leucocyma* | Leaves | High (3) | High (3) | High[b,c] (−3) | Low-Moderate (3) |
| *Ategumia lotanalis* | Leaves | High (3) | Moderate[a] (2) | High[b,c] (−3) | Low (2) |

[a] Only observed on *M. calvescens* and based on host specificity of related insects.
[b] Based on field and/or laboratory observations.
[c] Based on published studies with same and/or related species.

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# Giving dyer’s woad the blues: encouraging first results for biological control

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*Summary*

Dyer’s woad, *Isatis tinctoria* L. (Brassicaceae), has been cultivated since Roman times throughout Europe for the blue indigo dye extracted from its leaves and was introduced by early colonists into North America. Today, it is a declared noxious weed in ten western US states. A literature survey for insects, mites and pathogens associated with dyer’s woad revealed several biological control candidates. Three were found in 2004 during preliminary field surveys in Switzerland and Germany: *Ceutorhynchus rusticus* Gyllenhal and *Aulacobaris fallax* H. Brisout, both root-mining weevils, and *Psylliodes isatidis* Heikertinger, a shoot-mining flea beetle. Results of host-specificity tests conducted at CABI Europe-Switzerland are particularly promising for *C. rusticus*, a very damaging species able to kill overwintering rosettes. Results of additional host-specificity tests with *P. isatidis* are necessary to decide whether it is worth continuing with this species, while *A. fallax* is not specialized enough to be further considered. In 2006, additional field surveys were conducted in Turkey, Bulgaria, Romania and Kazakhstan to find new candidates. Based on the material identified thus far, two species are of interest, a flea beetle preliminarily identified as *Psylliodes sophiae* var. *tricolor* Weise and a root-mining weevil preliminarily identified as *Aulacobaris* near *fallax*. For both species, rearing colonies were established in Switzerland, and methods for host-specificity tests were developed. A literature survey revealed 62 species to be associated with dyer’s woad in Europe. Of the ten species only described from dyer’s woad (*I. tinctoria*) or closely related *Isatis* species, four are of particular interest, *viz*. the root-mining weevil *Aulacobaris licens* Reitter, an as-yet undescribed *Lixus* sp. and the two seed-feeding weevils *Bruchela exigua* Motschulsky and *Ceutorhynchus peyerimhoffi* Hustache. Surveys will be conducted in 2007 to find at least two of these four species, and investigations on already available agents will continue.

***Keywords***: *Isatis tinctoria*, weed biological control, host range testing.

## Introduction

Dyer’s woad, *Isatis tinctoria* L. (Brassicaceae), is a winter annual, biennial or short-lived perennial mustard of Eurasian origin. The original distribution of dyer’s woad in Europe and Eurasia is difficult to reconstruct because it has been widely distributed and grown by humans as a dye crop since Roman times. It was introduced to North America by early colonists as a textile dye crop and then accidentally spread as a contaminant of crop seed (Hegi, 1986; McConnell *et al*., 1999). Today, dyer’s woad is a declared noxious weed in ten western US states (USDA-NRCS Plants National Database, http://plants.usda.gov; Invaders Database, http://invader.dbs.umt.edu). It was estimated that dyer’s woad reduced crop and rangeland production in Utah by $2 million in 1981, and its infestation doubled there within 10 years (Evans and Chase, 1981, in McConnell *et al*., 1999). Unlike many other mustard weeds, dyer’s woad does not depend on disturbance to establish and can readily invade and dominate well-vegetated rangeland sites.

In spring 2004, an initiative was started by a consortium of southeastern Idaho and Utah counties, administered through the Hoary Cress Consortium (Dr Mark Schwarzlaender, University of Idaho) to investigate the potential for classical biological control of dyer’s woad. During preliminary surveys conducted by CABI

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Europe-Switzerland in 2004, three species were prioritized as biological control candidates, and investigations started.

Here we present data on the biology and host-specificity testing of the three species under study, as well as a list of additional candidates resulting from extensive literature and field surveys conducted in 2006.

## Biological control candidates studied since 2004

One flea beetle and two weevil species were found during preliminary surveys in southern Germany and southern Switzerland in 2004, and host-specificity tests were started in 2005. The current test plant list for dyer's woad comprises about 100 plant species and varieties, nearly two thirds of which are native to North America (Wilson and Littlefield, 2006).

### *Aulacobaris fallax* H. Brisout (Coleoptera: Curculionidae)

Females of this univoltine weevil lay eggs from the end of March until early summer into the petioles of dyer's woad or directly into the root. Larvae mine in the petioles, root crown and root of dyer's woad, in which they also pupate. Heavily infested plants are visibly stunted and may die (Hinz *et al*., 2004).

In no-choice oviposition and development tests conducted with 39 test plant species, mining and/or larvae were detected in 16 plant species, 12 of which are indigenous to North America. Subsequently, these plants were exposed in multiple-choice field cage tests. Apart from dyer's woad, mines or larvae were found in eight test plant species, all native to North America (Hinz *et al*., 2005, 2006). Although in five cases only, one or two of the plants offered were attacked, results indicate that the range of plants *A. fallax* females accept for oviposition and on which larvae are able to develop is broader than expected and includes several North American species. In conclusion, *A. fallax* is not regarded as specific enough for consideration as a biological control agent for dyer's woad.

### *Ceutorhynchus rusticus* Gyllenhal (Coleoptera: Curculionidae)

Females of this weevil lay eggs from September throughout winter until early spring. Eggs are mostly laid directly into the leaf surface under the epidermis. Larvae feed in the petioles and later in the root crown. Mature larvae leave the plants to pupate in the soil, and adults of the next generation start to emerge from the end of May onwards. Newly emerged weevils aestivate during July and August and resume feeding at the beginning of September (Hinz *et al*., 2004). The main shoot of heavily attacked plants often dies, which easily distinguishes attacked plants in the field.

During no-choice oviposition and development tests, 45 test species were exposed, including 24 native to North America. Of the 43 species for which results (adult emergence) are available, adults thus far have only emerged from three test species, *viz*. *Arabis holboellii* Hornemann, *Schoenocrambe linifolia* Nutt., and *Stanleya viridiflora* Nutt. However, while an average of 8.1 ± 1.5 adults emerged from dyer's woad (control) plants, only an average of 0.5 ± 0.5 to 1.5 ± 0.6 adults emerged from these non-target test plants. In subsequent choice tests with two of the species using five replicates each, no attack occurred on *A. holboellii*, and *S. linifolia* sustained attack at a lesser degree than dyer's woad (Hinz *et al*., 2005, 2006). Hoffmann (1954) and Freude *et al*. (1983) each listed *I. tinctoria* as the only host plant for *C. rusticus*. Although *C. rusticus* appears to have been collected previously from *Rorippa palustris* (L.) Besser (B. Korotyaev, unpublished data), we did not find attack on three other *Rorippa* species (*Rorippa amphibia* L., *Rorippa sinuata* Nutt. and *Rorippa sylvestris* (L.) Bess.) that we tested (Hinz *et al*., 2006).

In conclusion, *C. rusticus* is regarded as a very promising biological control candidate for dyer's woad. We will continue host-specificity tests in 2007 and are also planning to conduct an experiment to quantify the impact of *C. rusticus* on dyer's woad.

### *Psylliodes isatidis* Heikertinger (Coleoptera: Chrysomelidae)

In autumn, females of this flea beetle lay eggs in the soil close to the root crown of the host plant. Larvae hatch in early spring and feed in the developing shoots of dyer's woad. Mature larvae leave the plants to pupate in the soil, and adults of the subsequent generation emerge during June. After a brief feeding period, the beetles aestivate. This species shows good potential for rapid population increase and at high densities can kill shoots or whole plants (Hinz *et al*., 2004).

During no-choice larval transfer and development tests with nine test species, adult flea beetles emerged from four. However, a lower proportion of larvae successfully developed into adults on test plants (3–12%) compared to control (dyer's woad) plants (28%). In a subsequent multiple-choice field-cage test exposing 14 test species together with dyer's woad, adult beetles emerged from nine test species. However, while an average of 46 adults (maximum 130) emerged from individual dyer's woad plants, only 0.3 to 3.6 emerged from test species (Fig. 1). We assume that the density of adults released into the field cage was artificially high (considering the high number of adults that emerged from controls), and plants may have been placed too close to each other (Hinz *et al*., 2005). A second multiple-choice field cage test will be established in autumn 2006 releasing fewer beetles and exposing fewer test plants (Hinz *et al*., 2006). Depending

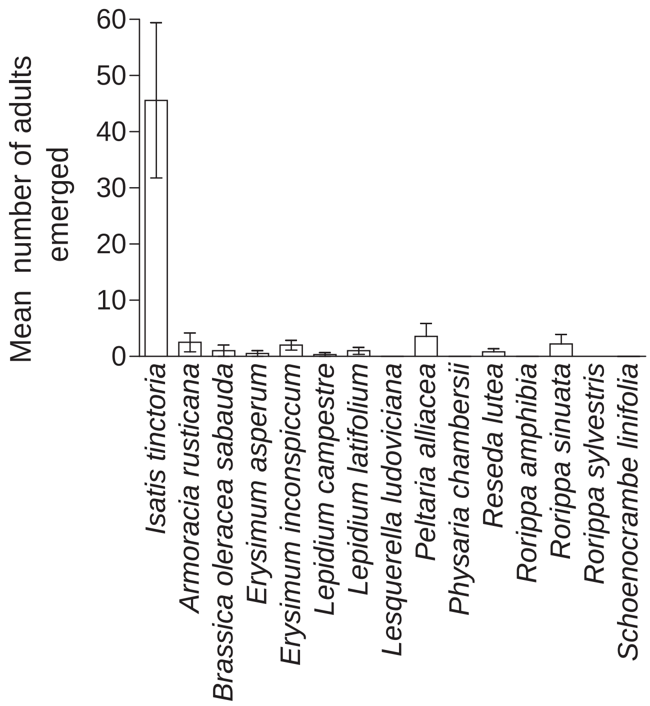


**Figure 1.** Mean number of *Psylliodes isatidis* adults emerged per plant during a multiple-choice field-cage test established at CABI Europe-Switzerland in September 2005.

on results obtained in summer 2007, we will make a decision whether it is worth continuing work with *P. isatidis*.

## New biological control candidates

Emphasis in 2006 was placed on field surveys in parts of the likely area of origin of dyer's woad with the aim to find additional candidates as well as to collect plant samples to investigate the genetic variability of the genus *Isatis* in Eurasia. Five field trips were conducted, three to central and northeastern Turkey, one to Romania and Bulgaria and one to Kazakhstan. Twenty-two field sites of dyer's woad were found. At each site, adult insects were sampled, plants were dissected, and plant parts (leaves, shoots, seeds, and roots) with immature insect stages or signs of mite or pathogen attack were collected and taken back to the lab. Immature stages were reared to adulthood and sent, together with field-collected adults, to taxonomists for identification. Combining data of the last 3 years, we have reared or sampled 52 insect species, 33 of which are known to feed and/or develop on plants of the family Brassicaceae. Half of the species ($n$ = 17) are weevils (Curculionoidea), and most of these belong to the genus *Ceutorhynchus* Germar. Based on identifications available thus far, two species could be of interest, *viz*. a stem-mining flea beetle, preliminarily identified as *Psylliodes sophiae* var. *tricolor* Weise, and a root-mining weevil, *Aulacobaris* sp. near *fallax* (H. Brisout), which is similar to *A. fallax* but most likely a distinct subspecies or a different species.

Apart from field surveys, we also continued to collect information on insects, mites and fungi associated with dyer's woad in the literature as well as by contacting taxonomists and collaborators. Combined with previous data, 56 insect species were found plus two nematodes and four fungi associated with dyer's woad. About one third were weevils (Curculionidae and Urodontidae), followed by flea beetles (Chrysomelidae, Alticini; $n$ = 10). Ten species are only described from *I. tinctoria* or closely related *Isatis* species. Of these, four are of particular interest, *viz*. a root-mining weevil, *Aulacobaris licens* Reitter, an as-yet undescribed *Lixus* sp. and two seed-feeding weevils, *Bruchela exigua* Motschulsky (=*Urodon exiguus*), and *Ceutorhynchus peyerimhoffi* Hustache.

In the following, short descriptions of these new potential biological control candidates are given.

### *P. sophiae* var. *tricolor* Weise (Coleoptera: Chrysomelidae)

This flea beetle was found in Bulgaria, central Turkey and Kazakhstan and preliminarily identified as *P. sophiae* var. *tricolor*. Larvae mined in the root crown of dyer's woad. At several sites, large numbers of this species were observed, and larval mining can be quite damaging, causing stunting of shoots. Therefore, although *P. sophiae* var. *tricolor* is described as oligophagous

and should also occur on another brassica species (e.g. *Sisymbrium sophiae* L.) (Freude *et al.*, 1966), we decided to prioritize it as a candidate. Beetles collected in the field were overwintered in an incubator at 3°C between 8 November 2006 and 26 February 2007. Females started to lay eggs in March, a colony was established,and methods for host-specificity tests were developed. The main emphasis will be to investigate whether a host race exists that is specific to dyer's woad.

### *Aulacobaris* sp. pr. *fallax* (H. Brisout) (Coleoptera: Curculionidae)

This species was found in central Turkey. Similar to *A. fallax* that was collected in southern Germany (see above), the larvae mine in root crowns and roots of dyer's woad, in which they also pupate. However, this population's oviposition behaviour is slightly different, and slight morphological differences were found (B. Korotyaev, unpublished data). In addition, *A. fallax* has never been recorded from Turkey (B.A. Korotyaev and L. Gültekin, unpublished data). Specimens from Germany and Turkey might therefore belong to two different species or subspecies. We are currently establishing a colony and plan to conduct preliminary host-specificity tests with plant species that supported development of *A. fallax* in earlier tests. In addition, the morphology of specimens sampled in Turkey and Germany will be studied in detail.

### *A. licens* (Reitter) (Coleoptera: Curculionidae)

The root-mining weevil *A. licens* occurs principally on *Isatis glauca*, species from northeastern Turkey closely related to *I. tinctoria*. During a field trip at the beginning of April, some specimens were collected and brought back to the quarantine facility. Weevils readily fed and laid eggs on cut dyer's woad (*I. tinctoria*) shoots offered in cylinders, indicating that this plant is also part of the host range of *A. licens*. Provided that *A. licens* will also successfully complete larval development on dyer's woad, a colony will be established, and host-specificity tests will start in 2008.

### *Lixus* (*Compsolixus*) sp. (Coleoptera: Curculionidae)

Several specimens of this probably undescribed species have been collected only on *I. glauca* in northeastern Turkey, while a few more have been found in European collections, mainly from Turkey. Although most species of *Lixus* Fabricius are not monophagous, a seemingly narrow distribution of this species and its rarity in the collections indicate the possibility of a restricted host range, and it warrants a closer examination as a candidate for dyer's woad. The larvae are believed to be stem and/or root miners.

### *B. exigua* (Motschulsky) (Coleoptera: Anthribidae)

*B. exigua* (= *U. exiguus*) is a seed-feeding weevil. It occurs in the southern Ukraine and the northern Caucasus region and may be monophagous on dyer's woad (Korotyaev, 1988). We are planning a field trip to these regions in the second half of May to collect adults, start a colony in quarantine at CABI and begin preliminary host-specificity tests.

### *C. peyerimhoffi* Hustache (Coleoptera: Curculionidae)

*C. peyerimhoffi* is known from Algeria, Italy and Greece. Specimens found in southeastern Turkey are most probably *Ceutorhynchus isatidis* Colonnelli (E. Colonnelli, B. Korotyaev, L. Gültekin, personal communication). The larvae of this species develop in the seeds of dyer's woad and thus far have only been collected from *I. tinctoria* in Europe (E. Colonnelli, personal communication). However, the species was originally described based on specimens collected in Algeria on the closely related *Isatis djurdjurae* Coss. (Hustache, 1916; Peyerimhoff, 1919). A field trip is planned in 2007 to collect this species.

We are not currently working with a seed feeder as candidate, and dyer's woad spreads exclusively by seed; therefore, *B. exigua* and *C. peyerimhoffi* are promising choices as new candidates.

## Conclusions and outlook

Since the start of the project in 2004, three candidates have been investigated, two weevils and one flea beetle. The root-miner *C. rusticus* shows very good potential as a biological control agent for dyer's woad, and host-specificity tests are well advanced. While the second weevil, *A. fallax*, turned out to be not specific enough, additional multiple-choice tests with the flea beetle *P. isatidis* will reveal whether it can be further considered as candidate.

As a result of additional field and literature surveys conducted in 2006, six additional species, five weevils and one flea beetle, were prioritized as new candidates. Three of the agents have already been collected, while the other three species will be collected during planned field trips later in 2007 or in 2008.

In addition to surveys for natural enemies, plant material was collected for morphological and molecular analyses. In Eurasia, the genus *Isatis* L. is represented by roughly 50 species (Hegi, 1986), and various subspecies of *I. tinctoria* are recognized. Observations during field surveys confirm this information, with considerable variability in leaf shape and colour, seed shape and phenology of dyer's woad seen. A thorough revision of the genus would therefore urgently be needed, especially since this will directly influence sur-

vey areas and agent selection. Plant material collected was sent to Dr John Gaskin (USDA, ARS, NPARL, Sidney, MT), who has kindly offered to process the material.

In conclusion, prospects for the biological control of dyer's woad look very promising. After only 3 years, host-specificity tests with three insect species are well advanced, and we have identified at least six additional species as candidates. Geographic areas (Turkey and Caucasus) with a wide variety of apparently specialized feeders on *Isatis* spp. were identified and will be further surveyed for biological control candidates in the future.

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# Herbivores associated with *Arundo donax* in California

**T.L. Dudley,[1,*] A.M. Lambert,[1,2,*] A. Kirk[3] and Y. Tamagawa[1]**

*Summary*

The Old World grass, *Arundo donax* L. (giant reed), is a serious invader of California riparian areas, and its purported ecosystem impacts led to its consideration as a target for biological control development. However, the herbivore complex in the *Arundo* adventive range has not been characterized, so there is little information regarding insects that may hinder biological control efforts by interfering with the release of new agents or that could be promoted as augmentative biological control agents if they have a substantial impact on the target weed. Here we report the results of surveys in California, with emphasis on three presumably non-indigenous insects that inflict significant damage to the host. One is a shoot-boring wasp, *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae), with a range limited to southern California and that damages shoots generally less than 1 cm in diameter. A shoot fly, *Cryptonevra* sp., is also associated with shoot damage and often mortality of secondary stems. A third herbivore, the aphid *Melanaphis donacis* (Passerini), is widespread in the southern and central parts of the state but has less apparent impact to the host. *T. romana* and *Cryptonevra* sp. are currently candidates for biological control development and introduction from overseas locations. Their established presence in California suggests that efforts could be revised to focus on documentation of host ranges and impacts under field rather than in quarantine conditions, in anticipation of future re distribution in North America.

***Keywords***: biological control, *Cryptonevra*, *Melanaphis donacis*, *Tetramesa romana*, giant reed; herbivore.

## Introduction

*Arundo donax* L. (giant reed) may be the most destructive invader of California riparian areas, displacing native vegetation, transpiring excessive groundwater, posing erosion and wildfire risks and providing poor wildlife habitat (Bell, 1994; Dudley, 2000; Kisner, 2004). Few arthropods appear to be associated with *Arundo* in California (Herrera and Dudley, 2003; Kirk *et al*., 2003), and most are using it as opportunistic structural habitat rather than as a food source. A variety of herbivores is found in the region of origin (from the Mediterranean Basin and across southern Asia), and the level of herbivore impacts is considered to be much greater than in California or other areas where *Arundo* is invasive (Kirk *et al*., 2003). Classical biological control is being developed using several candidate agents from Eurasia (Kirk and Widmer, 2004), but implementation is not anticipated for at least several years.

A standard element of a biological control programme should involve documentation of herbivores attacking the target weed in its adventive range to determine if new agents are needed or if effects of existing herbivores can be enhanced, as well as to evaluate the potential for interference with introduction of new agents (Harris, 1975; Olckers and Hulley, 1995). As part of the programme to build the ecological framework for justifying *Arundo* biological control, we are characterizing and comparing *Arundo* herbivores and associated plant condition in California and Southern Europe. Early investigations indicated that several non-indigenous insects, including a shoot-boring wasp of the family Eurytomidae, are present in California. The wasp was subsequently identified as *Tetramesa romana* (Walker), a widespread Mediterranean species and a primary candidate in the *Arundo* biological control programme. Members of this genus are highly host

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specific (Al-Barrak *et al*., 2004), which makes them particularly suitable for biological control.

Our initial objective is to determine the geographic extent of this and other current *Arundo* herbivores in California and to quantify their efficacy against this host weed. The larger objective is to increase the impact of existing herbivores through augmentative measures and/or to distribute them more widely to provide more extensive host suppression. We are currently evaluating their host specificity and appropriateness for mass rearing and redistribution.

## Materials and methods

To examine *Arundo* herbivore distributions in California, we conducted monthly insect surveys of *Arundo* stands on the Santa Clara River and less frequent (once or twice during study period) extensive surveys of *Arundo*-infested areas throughout the southwestern US (Table 1). Line transects 100 m long were established within *Arundo* vegetation, and samples were collected from 0.5 $m^2$ quadrats placed at 10-m intervals along transect lines. As of April 2007, a total of 994 *Arundo* shoots within all plots surveyed were cut at the soil surface and bundled together for transport back to the laboratory, where they were stored at 8°C until processed. Primary or main shoots and side shoots or secondary shoots were examined separately. Shoot lengths and diameters were recorded, and shoots were visually examined for herbivores and evidence of herbivore damage. These were then split lengthwise and examined for internal feeders. After dissection, shoots were dried (2 days at 55°C) to determine dry weight biomass. All recovered insects were sent to the USDA European Biological Control Laboratory in Montpellier, France for identification, and voucher specimens were deposited in the Santa Barbara Museum of Natural History, Santa Barbara, CA. Plots were also used to determine shoot density and biomass per unit area for analysis of plant growth differences between native and introduced ranges and evaluation of impacts of biological control agents after release and establishment.

Plant use by *T. romana* (oviposition, feeding and pupation sites) and potential impacts of infestation on *Arundo* were evaluated. We focused on this species because it is one of the primary agents being tested as a potential biological control (Kirk and Widmer, 2004). Shoot length, basal diameter and biomass of shoots and side shoots infested with *T. romana*, were compared with those of uninfested plants. Main shoot and side shoot data were analyzed separately using Student's *t* test.

## Results and discussion

No native insect herbivores were found using *Arundo* as a significant food source, in contrast to prior surveys that show numerous arthropods using this plant for non-consumptive purposes (Herrera and Dudley, 2003). Two non-native insects and another unidentified (but potentially non-native) insect were recovered from *Arundo* shoots during sampling. An aphid, *Melanaphis donacis* (Passerini), was found throughout the sampling range (except very northern California) with greatest abundance in coastal *Arundo* populations. Aphids feed primarily on the apical shoots and less mature, distal leaves, and although reaching high population densities in early spring in some locations, only minor damage was observed on plants in one location. Coccinellids were abundant on *Arundo* shoots several weeks after peak aphid densities and may have been responsible for the decrease in aphids by late May - aphids were often not present during sampling in July through December. We did not quantify aphid densities during surveys for several reasons; there was a lack of any visible aphid

**Table 1.** Sampling locations (river systems) by county, sampling intensity, and insects recorded in California.

| County | # Sampling sites | Insects |
|---|---|---|
| Alameda | 1 | *Melanaphis donacis* (Passerini) |
| Humboldt | 2 | None |
| Imperial | 2 | *Melanaphis donacis* |
| Inyo | 2 | None |
| Kern | 1 | None |
| Los Angeles | 4 | *Melanaphis donacis*, *Tetramesa romana* (Walker) |
| Mendocino | 2 | None |
| Monterey | 5 | *Melanaphis donacis* |
| Orange | 2 | *Melanaphis donacis*, *Tetramesa romana* |
| Riverside | 3 | *Melanaphis donacis*, *Tetramesa romana* |
| San Bernardino | 3 | *Tetramesa romana* (one site) |
| San Diego | 5 | *Melanaphis donacis*, *Tetramesa romana* |
| San Luis Obispo | 2 | *Melanaphis donacis* |
| Santa Barbara | 7 | *Melanaphis donacis*, *Cryptonevra* sp., *Tetramesa romana* |
| Santa Clara | 1 | *Melanaphis donacis* |
| Ventura | 8 | *Melanaphis donacis*, *Cryptonevra* sp., *Tetramesa romana* |
| Yolo | 1 | *Melanaphis donacis* |

feeding effect, aphids were often dislodged from stems during stem sampling and collection, aphid movement reduced accurate measurements, and the time and labor-intensive nature of counting aphids would have substantially reduced our ability to adequately sample *Arundo* populations throughout the state.

Dipteran larvae were recovered from over 80% of *Arundo* shoots in one area on the floodplain terrace in the Santa Clara River, Ventura County, CA. We infrequently found similar damage throughout this river system and the nearby Ventura River. Several larvae feed together in the upper nodes on main shoots of plants, and feeding damage resulted in atypical 'witches broom' shoot growth with 25% stem mortality in infested shoots (Fig. 1). The 'witches broom' shoot growth was evident in most shoots covering a 2-ha radius. We did not examine these damaged shoots for bacterial disease, which can cause this type of deformity; however, we assume that feeding damage on the primary shoot promoted secondary shoot production, as similarly occurs in stems with wind-damaged primary shoots. The gregarious larvae were associated with 'hour glass' shoot damage (Fig. 2) and may be an inquiline species that feeds on microbes that colonize damage from another fly species (A. Kirk, personal observations). Chloropid flies (*Cryptonevra* sp.) in the Mediterranean region primarily attack developing canes up to about 75 cm in length and cause similar 'hour glass' damage to what we observed. In addition, it or another species of Chloropidae attacks side shoots and/or leading shoots on taller canes and are often followed by several inquiline species - up to 18 inquiline spp. have been recorded from southern France (A. Kirk, unpublished data). Damage prevents shoots from elongating during growth and results in stunted shoots and a 'witches broom' appearance from increased side shoot production (Fig. 1). We are focusing our sampling efforts during the spring growing period when herbivores producing the initial shoot damage may be present. *Cryptonevra* sp. is a candidate for potential biological control introduction (Tracy and Deloach, 1998), so further documentation of its distribution and impacts in North America and verification that the species present here is the same as the Mediterranean taxon being tested by US Department of Agriculture-Agricultural Research Service (USDA-ARS) are essential for future agent selection.

The shoot-boring wasp, *T. romana*, was collected from *Arundo* populations throughout southern California (Fig. 3). Its exit holes and gall-like formations produced during larval feeding are evident on primary

**Figure 1.** *Arundo donax* L. shoot infested with *Cryptonevra* sp. in the Santa Clara River, CA (*arrow*). Note the atypical 'witches broom' appearance of the infested shoot relative to the other, uninfested shoots.

**Figure 2.** *Arundo donax* L. shoot damage caused by *Cryptonevra* sp. feeding. The *arrow* points to the characteristic 'hourglass' damage.

and secondary shoots. We have observed oviposition on shoots both in greenhouse cultivation and at field sites (Fig. 4). Shoot infestation was variable (range: 0% to 80%) with a mean of 23.1 ± 4.4% (±SE) shoots infested across all sites sampled ($n$ = 994). However, in March 2007, we observed about 2.5 ha of an *Arundo* stand near the Santa Clara River (Ventura Co.) with ca. 99% of side shoots infested and killed. A recent survey at this same location suggests that infestation levels of current-year shoots are still high.

Wasp densities are highest on smaller diameter main shoots (primarily new shoots) and side shoots of larger, mature shoots (Fig. 5). Wasp densities can exceed 35 individuals on a main shoot and six individuals on a

**Figure 3.** Distribution of *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae) in California as of April 2007.

**Figure 4.** *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae) ovipositing in an *Arundo* side shoot. *Bar* represents 2mm.

side shoot, but average 4.9 ± 3.6 individuals per main shoot and 3.4 ± 1.9 per side shoot. Infested main shoots were shorter and smaller in diameter than uninfested shoots (shoot height, $t = 3.02$, $df = 315$, $p = 0.003$; shoot diameter, $t = 3.93$, $df = 315$, $p < 0.001$), but biomass was not significantly different between infested and uninfested shoots. Infested side shoots were thinner than herbivore free shoots ($t = 2.0$, $df = 197$, $p = 0.05$). Side shoot height and biomass were not significantly affected by wasp infestation. In France, *T. romana* infests shoots over a broader diameter range (A. Kirk, personal observation); *Arundo* shoots are also, on average, thinner and shorter than in North American populations (A. Lambert, unpublished data).

Biomass reduction in infested shoots could not be inferred from these data for two reasons: (1) extreme

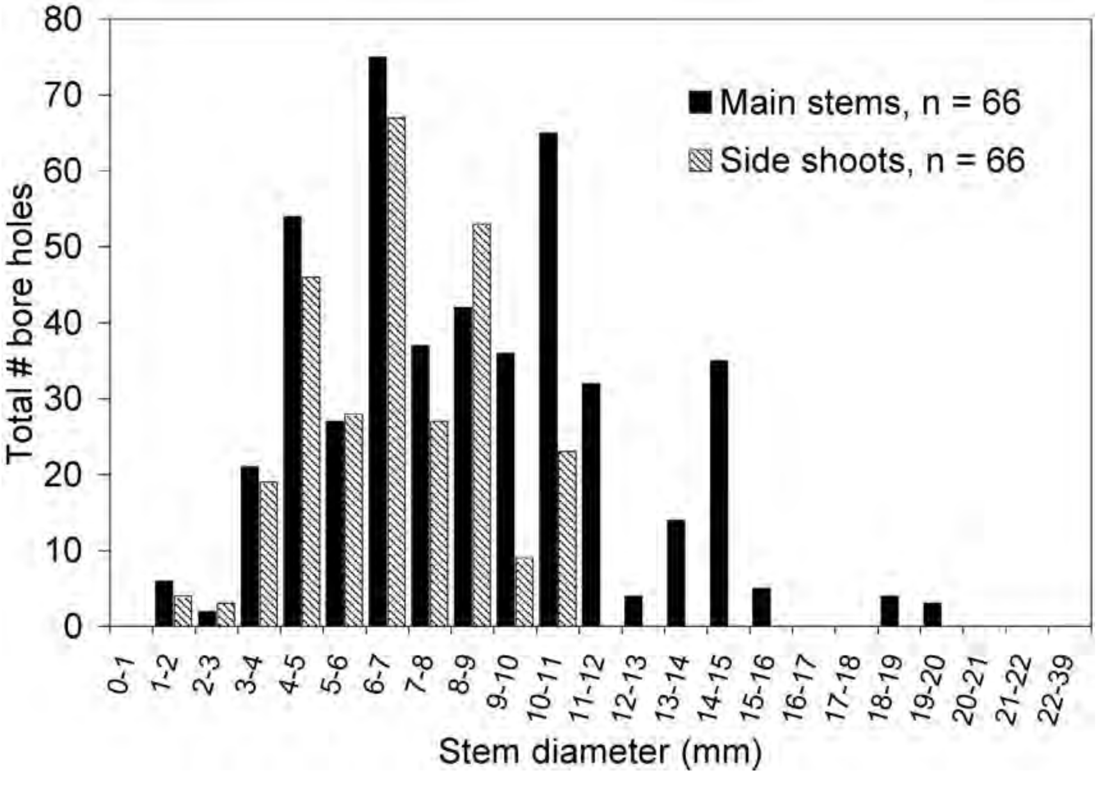


**Figure 5.** Distribution of emergence holes of *Tetramesa romana* (Walker) over the stem density range (mm) of *Arundo donax* L. main and side shoots.

variability in measures of morphological character (shoot length, diameter and biomass) even with substantial replication and (2) wasps colonize primarily new shoots and secondary shoots, which have lower biomass than mature and main shoots, respectively (Spencer *et al*., 2006). Therefore, wasp impact may be less noticeable on young shoots relative to that of an herbivore attacking the larger mature shoots. The frequency of damage but lack of substantial impact of *T. romana* to *Arundo* populations in our surveys does not necessarily reflect an absence of impact for this species. It is anticipated that this herbivore, in association with other agents such as the rhizome-feeding scale insect, *Rhizaspidiotus donacis* (Leonardi), may inflict greater damage to host plants (A. Kirk, personal observations).

Further surveys are necessary to accurately determine the full extent and limits of the *T. romana* distribution and its potential to infest a greater proportion of shoots. For example, as our sampling size increases, the relationship between wasp density and main and side shoot mortality becomes stronger. We are currently evaluating above- and belowground biomass relationships of infested and uninfested plants in a common garden experiment to elucidate the effect of herbivory on resource allocation. Experiments are also being conducted by the USDA-ARS to determine the impact of *T. romana* on water use by *Arundo* (J. Goolsby, USDA, Weslaco, TX, personal communication).

Genetic comparisons are underway in collaboration with D. Kazmer and W. Jones, USDA-ARS, Sidney, MO, and European Biological Control Laboratory (EBCL), using newly eclosed females collected throughout California and matching with wasps collected in the Mediterranean region to determine the origin of California *T. romana*. It is unclear at this time whether this wasp is a recent introduction in California and is still in its establishment phase, in which case its potential (future) range may be much greater. Alternatively, if *T. romana* has been established for a long period, then dispersal may have achieved the full extent of its potential range, and the likelihood for redistribution is poorer. Wasps have been found throughout the watersheds of Ventura County, whereas stands in other counties, particularly at the northern and southern extents of its apparent range, are often colonized more heterogeneously with uninfected streams in moderately close (e.g. <10 km) proximity to colonized stands. This may indicate that the colonization process is, as yet, incomplete. Patchy distributions could also represent reduced physiological compatibility with climate regimes at the limits of the *T. romana* distribution. Such a distribution in the warm- temperate coastal region of California is not dissimilar to other organisms introduced for biological control of other California pests, e.g. parasitoid *Aphytis* spp. against the citrus red scale (Rosen and DeBach, 1978, 1979). Furthermore, it is not yet clear whether there may have been the co-introduction of *T. romana* parasitoids (A. Kirk, unpublished data) and subsequent population regulation, which could also explain the variation in attack rate observed over *T. romana*'s distribution in California.

The unintentional introduction of *T. romana* and other apparent specialist herbivores to North America provides the opportunity to conduct host-specificity trials and other experimental studies in the field, which will provide instructive ecological information less easily obtained under quarantine conditions. Plant growth and insect behavior are often constrained or atypical in highly controlled environments (Blossey *et al*., 1994a,b), so results in open-field settings are expected to be more representative of natural responses. We suggest that an *Arundo* biological control programme based on development of 'California' *T. romana* and *Cryptonevra* sp. as augmentative biological control agents for local population enhancement and/or re-distribution in North America should be carried out in parallel with foreign importation and further quarantine testing. Host-range testing should be continued to validate host specificity prior to agent movement, but conducting this work with insects already established in North America would enhance the ecological validity of the tests and conserve financial resources. This survey of California *Arundo* herbivores also validates the advice of Harris (1975) and others that biological control be developed as a logical progression, with evaluation of weed ecology and associated herbivores in the invasive range before importing new organisms.

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# Which species of the thistle biocontrol agent *Trichosirocalus* are present in New Zealand?

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*Summary*

*Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae), nodding thistle crown weevil, was introduced to New Zealand in 1989 for classical biological control of *Carduus nutans* L. (nodding thistle). Later, it was introduced from New Zealand to Australia. In 2002, a revision of the species concluded that *T. horridus* was in fact a complex of three species, with distinct host plant genus preferences: *T. horridus*, *Trichosirocalus mortadelo* Alonso-Zarazaga and Sánchez-Ruiz, and *Trichosirocalus briesei* Alonso-Zarazaga and Sánchez-Ruiz with preferences for *Cirsium*, *Carduus* and *Onopordum* thistles, respectively. In the revision, crown weevils on *Carduus* thistles in Australia were identified as all *T. mortadelo*, sourced from New Zealand. This suggests that the original introductions into New Zealand were wholly or partly *T. mortadelo*. A survey conducted to confirm which species of *Trichosirocalus* are present in New Zealand shows that all adults collected here are *T. horridus* regardless of whether the source host was *Cirsium vulgare* (Savi) Tenore or *C. nutans.* This presents two paradoxes: firstly, that *T. mortadelo* was collected from New Zealand and shipped to Australia but has not been found in our surveys, and secondly, that *T. horridus* does not show distinct preference for *Cirsium* thistles in New Zealand as reported elsewhere.

***Keywords***: *Trichosirocalus horridus*, *T. mortadelo*, *Carduus nutans*, *Cirsium vulgare*, *Onopordum acanthium*.

## Introduction

The thistle biological control agent *Trichosirocalus horridus* (Panzer) was first introduced to New Zealand in 1984 and was released in the Canterbury plains of the South Island (Jessep, 1989b). By 1989, established populations were available for distribution to further sites in both the North and South Islands, and the weevil has been considered established in New Zealand ever since. The weevils introduced to New Zealand are originally from *Carduus nutans* L. from Neuenburg, Germany, and were established on *C. nutans* in Canada (Agriculture Canada, Regina Station, SK) prior to their introduction (Jessep, 1989a, 1989b; Julien and Griffiths, 1998; P. Harris, personal communication).

Preliminary trials in New Zealand soon after the weevil's establishment suggested it readily attacked the three *Carduus* spp. present in the South Island, *C. nutans*, *Carduus pycnocephalus* L. and *Carduus tenuiflorus* Curtis (the fourth *Carduus* sp., present only in New Zealand's North Island, *Carduus acanthoides* L., was not tested), and two *Cirsium* spp., *Cirsium vulgare* (Savi) Tenore and *Cirsium palustre* (L.) Scopoli. It did not attack *Cirsium arvense* (L.) Scopoli, *Silybum marianum* (L.) Gaertner and *Onopordum acanthium* L. (Jessep, 1989b). The damage caused by weevil attack to both *Carduus* and *Cirsium* spp. in these trials was substantial.

In 1992, *T. horridus* from New Zealand was introduced to Australia for *C. nutans* control, and releases were initiated in 1993 (Woodburn, 1997). At the same time, scientists from Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australia

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were engaged in research in Europe towards a biocontrol programme for *Onopordum* spp. thistles (Briese *et al.*, 1994). One of the highly prioritized biocontrol agents was identified as *T. horridus*, partly because it had already been used successfully in Virginia for controlling the related *C. nutans* thistle (Kok, 1986, cited by Briese *et al.*, 1994). The weevils were introduced from Europe into quarantine in Australia, where it was noticed that they exhibited some consistent non-similarities to the *T. horridus* introduced from New Zealand for *C. nutans* control (T. Woodburn, personal communication). Specimens were sent to a taxonomist (M. Alonso-Zarazaga), who re-described the species and revealed that, what was until then regarded as one species, *T. horridus*, was in fact a complex of three species (Alonso-Zarazaga and Sánchez-Ruiz, 2002). The samples sent from Australia included specimens of the *Onopordum* specialist from quarantine and specimens from the population established on *C. nutans* near Canberra. The latter are considered progeny of the weevils introduced from New Zealand (Alonso-Zarazaga and Sánchez-Ruiz, 2002). The European samples from *Onopordum* in quarantine in Australia were identified as a new species, *Trichosirocalus briesei* Alonso-Zarazaga and Sánchez-Ruiz, with trophic linkage to the thistle genus *Onopordum* (larvae and adults did not survive on *Carduus* spp. and *Cirsium* spp.). Specimens from *C. nutans* from the Canberra region were, too, identified as a new species, *Trichosirocalus mortadelo* Alonso-Zarazaga and Sánchez-Ruiz, associated with thistles of the genus *Carduus*, mainly *C. nutans* (Alonso-Zarazaga and Sánchez-Ruiz, 2002). It was assumed that the weevils introduced from New Zealand to Australia were either *T. mortadelo* or a mixture of *T. mortadelo* and *T. horridus*. The species *T. horridus* was now associated with thistles of the genus *Cirsium* (Alonso-Zarazaga and Sánchez-Ruiz, 2002).

In Australia, efforts to establish weevils originating from New Zealand on *Cirsium* spp. were unsuccessful (A. Swirepick, personal communication), which settles with their identification as *T. mortadelo*, the *Carduus* specialist. In New Zealand, however, the weevils occur on both *Carduus* spp. and *C. vulgare*, although their effect on *C. vulgare* populations is, for the most part, somewhat poor. *Trichosirocalus* spp. are extremely attractive biocontrol agents for weeds that exhibit a long-lived seed bank, such as *C. nutans* and *Onopordum* spp., as they reduce the plants' vigour such that they either produce less seeds or even die prior to any seed production; and they also reduce plant biomass at the time that lapses until seed reserves in the soil are depleted (Briese, 2006). It was therefore desirable to find out whether the non-satisfactory control of *C. vulgare* in New Zealand was attributed to *T. horridus*, the *Cirsium* specialist, not being present here. In 2006, a survey was conducted in New Zealand to record which thistle species are attacked by which *Trichosirocalus* spp.

## Methods and materials

A field survey was initiated in autumn 2006 and lasted to the end of summer 2007. Regional Councils staff visited release sites in different parts of the country, collected weevil specimens and, where possible, estimated whether thistle populations decreased since the weevil's introduction into their region. All thistle species present at each site were noted, as well as the relative abundance of damaged rosettes (which can be readily distinguished by the black frass secreted by the feeding larvae). Where weevils were found on more than one thistle species at one site, they were collected separately for each thistle species. Sample size varied between sites, depending on the difficulty to obtain specimens. All weevils were preserved in 70% ethanol prior to identification.

For identification, all weevils were dissected under a stereo microscope. Their gender was recorded, and male's aedeagi were examined against the key provided in Alonso-Zarazaga and Sánchez-Ruiz (2002). All specimens were then mounted and deposited at the University of Canterbury arthropod collection for future referencing.

## Results and discussion

Different responses from Regional Councils in different parts of the country resulted in large variation in the number of samples collected per region. Eight regions (three in the North Island and five in the South Island, Figure 1) and 51 sites were sampled, and 744 adults were dissected, 337 of which were males. In one region (Auckland), no weevils could be collected. All males in all samples were identified as true *T. horridus* (Table 1). Where estimated, *C. nutans* populations in New Zealand's South Island appear to have decreased since *T. horridus* introduction (Table 2).

Three main points can be highlighted: (1) there is no evidence for any species other than *T. horridus* being present in New Zealand (Table 1); (2) *T. horridus* readily attacks *Carduus*, *Cirsium* and *Onopordum* thistles (Tables 1 and 3); and (3) *C. vulgare* appears to be, for the most part, affected not as strongly as *C. nutans* and *O. acanthium* by the weevil (Tables 2 and 3). The latter two points are inconsistent with the recent re-description of the species (Alonso-Zarazaga and Sánchez-Ruiz, 2002). According to this description, *T. horridus* should be able to feed on *Carduus* spp. but should show preference for *Cirsium* spp., in particular to *C. vulgare*. In addition, it has been indicated that *T. horridus* might be seen on *Onopordum* spp. in mixed thistle stands but that it would not feed on it (M. Alonso-Zarazaga, personal communication).

The presence and feeding of *T. horridus* on *O. acanthium* in New Zealand is of special interest: in the early 1990s, not long after the weevil had been introduced to

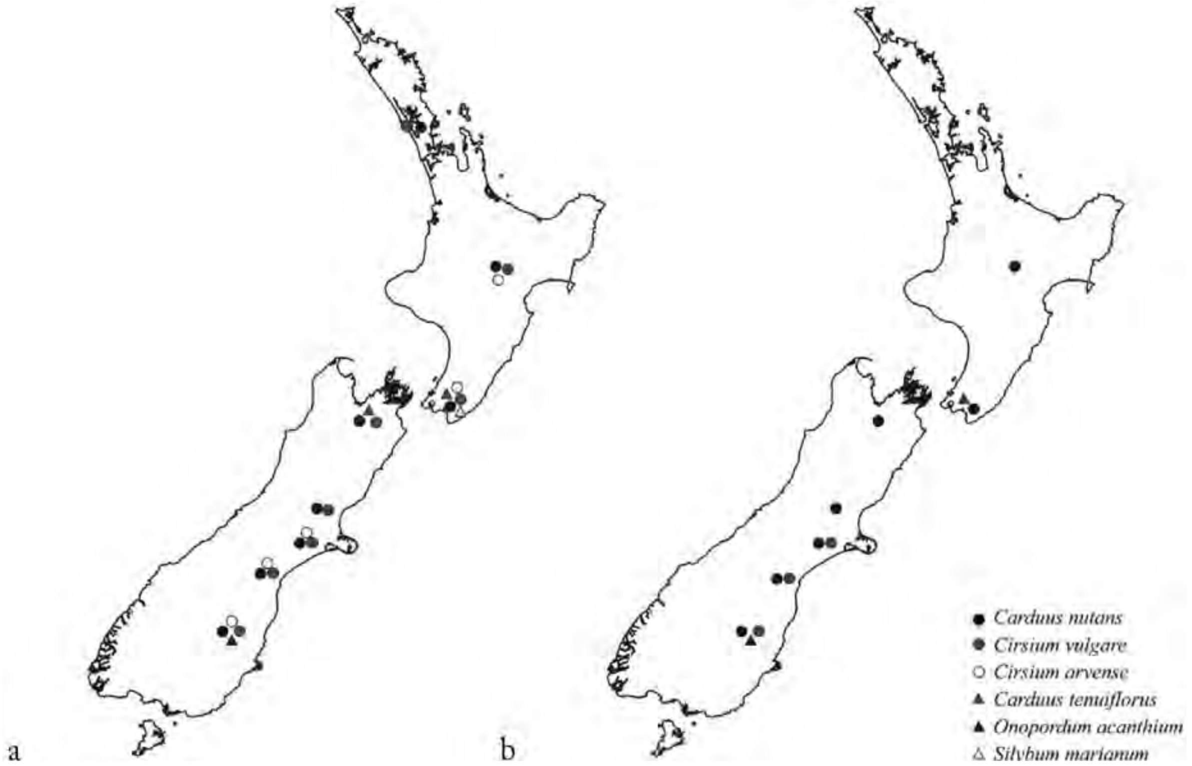


**Figure 1.** Regions of New Zealand visited in the survey with points marking: (a) thistle species present in each region and, (b) species from which weevils were collected.

**Table 1.** Geographic origin of *Trichosirocalus* spp. samples and species ID.

| Region | Number of sites sampled | Host thistle species | Number of males examined | Species ID[a] |
|---|---|---|---|---|
| **North Island** | | | | |
| Central NI | 3 | *Carduus nutans* | 20 | *Trichosirocalus horridus* |
| Greater Wellington | 1 | *Carduus nutans* | 8 | *Trichosirocalus horridus* |
| | 1 | *Carduus tenuiflorus* | 6 | *Trichosirocalus horridus* |
| **South Island** | | | | |
| Tasman | 1 | *Carduus nutans* | 20 | *Trichosirocalus horridus* |
| North Canterbury | 3 | *Carduus nutans* | 39 | *Trichosirocalus horridus* |
| Mid Canterbury | 2 | *Carduus nutans* | 19 | |
| | 2 | *Cirsium vulgare* | 13 | |
| South Canterbury | 29 | *Carduus nutans* | 158 | |
| | 5 | *Cirsium vulgare* | 12 | *Trichosirocalus horridus* |
| Central Otago | 1 | *Carduus nutans* | 15 | *Trichosirocalus horridus* |
| | 1 | *Cirsium vulgare* | 5 | *Trichosirocalus horridus* |
| | 2 | *Onopordum acanthium* | 22 | *Trichosirocalus horridus* |
| Total | 44[b] | | 337 | *Trichosirocalus horridus* |

[a] Based on male aedeagus; identified using the key provided in Alonso-Zarazaga and Sánchez-Ruiz (2002).
[b] In some sites, weevils were collected from more than one thistle species.

**Table 2.** Estimates of changes to *Carduus nutans* and *Cirsium vulgare* populations since the introduction of *T. horridus.*

| | Number of sites where populations were | | |
|---|---|---|---|
| | Estimated to have declined | Estimated to have not declined | Not estimated (unknown site history) |
| *Carduus nutans* | | | |
| North Island | 3 | 3 | 0 |
| South Island | 16 | 6 | 17 |
| *Cirsium vulgare* | | | |
| North Island | 1 | 1 | 3 |
| South Island | 2 | 2 | 18 |

New Zealand and had established, T. Jessep (who was responsible for its introduction and distribution), realizing the weedy status of *Onopordum* spp. in Australia, decided to establish the weevil on *O. acanthium* in New Zealand as a measure to prevent this thistle from becoming weedy here (M. Turner, personal communication). *O. acanthium* (the only *Onopordum* spp. present in New Zealand) is not common and can mainly be found in Central Otago. A site was located in Central Otago, hosting an isolated dense *O. acanthium* stand, with no *C. nutans* to be found at a radius of 10 km. In 1993, 1300 weevils, collected from *C. nutans*, were released at the site (M. Turner, personal communication). The weevils have established and have dramatically reduced *O. acanthium* density at the site over several years. Today, only few thistles are germinating, fewer reach the flowering stage, and ones that do flower show reduced height and vigour (R. Groenteman, personal observation). Moreover, with the loss of prickliness attributed to the weevil's larval feeding, the capitula appear palatable to the deer grazing on the farm, which further helps depleting the soil seed bank. The landowner assured us that he does not manage thistles on the farm by any other control means or land management practices. The weevils were never redistributed from that original site; yet we were able to collect them from another *O. acanthium* stand, about 50 km (aerial distance) away from the original site. At this distant site, part of the plants were high and vigorous and exhibited no weevil feeding signs, whereas others appeared much less vigorous, exhibited feeding signs, and hosted the weevil (R. Groenteman, personal observation). It seems that the weevils dispersed by themselves to the site, and their impact is beginning to be noticeable. Adults that

**Table 3.** Relative abundance of different thistle species in different regions of New Zealand and fraction of sites in which *Trichosirocalus horridus* adults and/or damaged rosettes were abundant.

| Region | Host species | Presence[a] | Relative abundance of thistle rosettes | Frequency of sites in which adult *T. horridus* and/or damaged rosettes were abundant |
|---|---|---|---|---|
| **North Island** | | | | |
| Auckland | *Carduus nutans* | 1/1 | moderately common | 0/1 |
| | *Cirsium vulgare* | 1/1 | moderately common | 0/1 |
| Central NI | *Carduus nutans* | 3/3 | very common | 1/3 |
| | *Cirsium vulgare* | 2/3 | moderately to very common | 0/2 |
| Greater Wellington | *Carduus nutans* | 2/2 | rare | 1/2 |
| | *Cirsium vulgare* | 2/2 | rare | 1/2 |
| **South Island** | | | | |
| Tasman | *Carduus nutans* | 1/1 | moderately common | 0/1 |
| | *Cirsium vulgare* | 1/1 | rare | 0/1 |
| North Canterbury | *Carduus nutans* | 3/3 | moderately to very common | 3/3 |
| | *Cirsium vulgare* | 2/3 | rare | 0/2 |
| Mid Canterbury | *Carduus nutans* | 3/3 | varies | 1/3 |
| | *Cirsium vulgare* | 3/3 | moderately common | 0/3 |
| South Canterbury | *Carduus nutans* | 30/30 | moderately common mostly | 21/30 |
| | *Cirsium vulgare* | 14/30 | rare to moderately common | 5/14 |
| Central Otago | *Carduus nutans* | 2/3 | moderately common | 2/2 |
| | *Cirsium vulgare* | 2/3 | rare to moderately common | 1/2 |
| | *Onopordum acanthium* | 2/3 | moderately to very common | 2/2 |
| NZ total | *Cirsium nutans* | 45/46 | | 29/45 |
| | *Carduus vulgare* | 27/46 | | 7/27 |

[a] Presence is expressed as the fraction of sites in which the thistle species was recorded, out of the total number of sites visited in each region.

were collected from *O. acanthium* at both sites were identified, again, as *T. horridus*. At the self-introduced site, adult weevils were also collected from *C. vulgare*, and these, too, were identified as *T. horridus* (Table 1).

The results from the survey thus far present two paradoxes: firstly, that *T. mortadelo* was collected from New Zealand and shipped to Australia but has not been found in our survey, and secondly, that *T. horridus* does not show a distinct preference for *Cirsium* thistles in New Zealand as reported elsewhere.

What, then, might the origin of *T. mortadelo* in Australia be? It appears that although CSIRO scientists have been engaged in searches for biocontrol agents in several European countries in the mid 1990s, the only *Trichosirocalus* sp. introduced into Australia as a result of this were the *Onopordum* specialist from Spain, later named *T. briesei*; and the only weevils released on *Carduus* thistles a few years earlier were of New Zealand origin (A. Sheppard, D. Briese, T. Woodburn, A. Swirepick, personal communication). If any *Trichosirocalus* spp. had been collected from *Carduus* thistles in Europe as part of these surveys, they would have been examined in quarantine only and not released (A. Sheppard, personal communication), whereas the *Carduus* weevils used in the revision were collected from established populations in Australia (D. Briese, personal communication).

It has been suggested that *T. mortadelo* may have disappeared from New Zealand or was out-competed by *T. horridus*. An examination of 30 specimens that were collected in South Canterbury in 1990, as part of a Lincoln University Master thesis, does not support this possibility. These specimens were collected at the original release site in New Zealand, where the weevils have been established the longest. This, most probably, was the site that sourced the weevils for the introduction to Australia. Sixteen of these specimens were males, and they were identified as *T. horridus*. Thus, it is hard to believe *T. mortadelo* was there at the time but was not represented in the sample.

A third paradox arises from the revision itself (Alonso-Zarazaga and Sánchez-Ruiz, 2002) and is supported by the personal communications surrounding this survey: that there is no evidence for *T. horridus* presence in Australia.

## Conclusions and outlook

To conclude, it appears that the *Trichosirocalus* spp. fauna in New Zealand consists of only one species, *T. horridus*, feeding on thistle hosts belonging to the genera *Carduus*, *Cirsium* and *Onopordum*. It is unclear why the weevils in New Zealand exhibit host utilization that is inconsistent with the recent revision. It also remains unclear where *T. mortadelo* in Australia originates from and why *T. horridus* is absent there. A similar survey in Australia would shed some light on these matters.

It is probably not desirable to re-introduce *T. horridus* from *C. vulgare* to achieve better control of this thistle, since the specialized populations may blend with the existing populations already well established on *Carduus*. However, it might be useful to harvest weevils from the few sites in New Zealand where they do well on *C. vulgare* and redistribute those. It is certainly unjustified to introduce the *Carduus* specialist, *T. mortadelo*, since *T. horridus* is well established on *C. nutans* and appears to have a significant negative effect on this weed.

## Acknowledgements

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# Bionomics and seasonal occurrence of *Larinus filiformis* Petri, 1907 (Coleoptera: Curculionidae) in eastern Turkey, a potential biological control agent for *Centaurea solstitialis* L.

**L. Gültekin,[1] M. Cristofaro,[2,3] C. Tronci[3] and L. Smith[4]**

*Summary*

We conducted studies on the life history of *Larinus filiformis* Petri, 1907 (Coleoptera: Curculionidae: Lixinae) to determine if it is worthy of further evaluation as a classical biological control agent of *Centaurea solstitialis* L. (Asteraceae: Cardueae), yellow starthistle. The species occurs in Armenia, Azerbaijan, Turkey and Bulgaria. Adults have been reared only from *C. solstitialis*. In eastern Turkey, adults were active from mid-May to late July and oviposited in capitula (flower heads) of *C. solstitialis* from mid-June to mid-July. In the spring, before females begin ovipositing, adults feed on the immature flower buds of *C. solstitialis*, preventing them from developing. Larvae develop in about 6 weeks and destroy all the seeds in a capitulum. The insect is univoltine in eastern Turkey, and adults hibernate from mid-September to mid-May.

***Keywords***: *Larinus filiformis*, *Centaurea solstitialis*, bionomics.

## Introduction

*Centaurea solstitialis* L. (Asteraceae: Cardueae), yellow starthistle, is an important invasive alien weed in rangelands of the western USA (Maddox and Mayfield, 1985; Sheley *et al*., 1999; DiTomaso *et al*., 2006). Although six species of insects have been introduced to the USA for biological control of this weed, there is still interest to find additional prospective agents (Balciunas, 1998; Smith, 2004a; Pitcairn *et al*., 2006). Greece and Turkey are considered to be the geographic centre of diversity of *C. solstitialis* (Wagenitz, 1975; Dostál, 1976). Recent explorations carried out in Eastern Turkey revealed the presence of *Larinus filiformis* Petri, 1907 (Coleoptera: Curculionidae), a weevil strictly associated with *C. solstitialis* (Cristofaro *et al*., 2002, 2006; Gültekin *et al*., 2006). *L. filiformis* was originally described from Arax Valley (Armenia) and is included in the Lixinae subfamily (Petri, 1907; Ter-Minassian, 1967). However, nothing is known about its biology. The goal of this work was to clarify this insect's life history, including host range, seasonal occurrence and geographic distribution in Turkey.

## Methods and materials

Field surveys and observations were conducted from early spring through summer during 2003. The goals were to collect live adults for biological experiments and to observe hibernation places, initiation of adult activity in the spring, adult feeding, mating, oviposition, larval feeding and development, host plants, oviposition preference, season of occurrence of different developmental stages and to collect associated parasitoids. The principal study sites were located in Bingöl

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Province (from 35 km north-east of Bingöl to 15 km west of Bingöl) and in Iğdır Province, (from 6 km east of Tuzluca to 7 km east of Iğdır). Both provinces are temperate regions. The Bingöl region is characterized by *Quercus* forest with open areas, including abandoned fields where *C. solstitialis* commonly occurs. Iğdır Province contains the Aras river valley and Ağrı mountain lowland area (Iğdır Plain). The Aras valley is quite desertified and eroded and dominated mainly by semi-desert vegetation. When we found a site with at least 100 *C. solstitialis* plants, we searched yellow starthistle plants for signs of *L. filiformis* (e.g. feeding and oviposition damage and presence of adults). During 1997 to 2006, while conducting a survey of *Larinus* biodiversity, the lead author recorded host plant associations in eastern Turkey by examining many plants in the tribe Cardueae (Asteraceae). Mature flower heads were collected and held to rear adult insects from known host plants.

# Results and discussion

## Insect morphology

Adult body length is 4.5 to 6.5 mm. The body is black, the scape, funicle and tarsi are chestnut-brown, and the tibia and apex of femur are brownish-black (Figure 1). Golden colour hair-like scales are scattered sparsely on pronotal disc and body. Elytra are clothed with bifurcate short whitish-grey scales, which are more dense on second to fourth intervals (where the first interval is the longitudinal stripe closest to the center of the insect when wings are folded) and on the lateral margins. The rostrum is cylindrical, weakly curved, 1.0 to1.5 times as long as pronotum and is distinctly longer and more shiny in females than males (Figure 1). Elytra are parallel sided at the basal half and then gradually curved toward the apex.

## Life history

First adult activity in the spring was recorded during the third week of May. By the end of May, most of the insects had emerged from hibernation. Early season field collections suggest that males become active earlier than females. Mating was not observed in the field until the end of May, but it continued throughout the adult life span. Adults feed on young buds, on the central growing tip of the plant and on leaves of *C. solstitialis*. Later in the season, they feed on the internal receptacle tissue and flower parts of immature and mature flower heads. Adults were active in the field from 19 May to 3 August 2003.

Based on our observations in the field and cage studies, three conditions were necessary to begin oviposition: temperature above 25°C, feeding for about 20 days and availability of mature *C. solstitialis* flower heads. In the field, oviposition was recorded from mid-June to mid-July. Females chewed an ovate hole with their rostrum in the lower part of mature, rarely pre-flowering flower heads and laid a single egg into the receptacle tissue. The oviposition hole is then closed with a secretion. Eggs hatched in about 13 days under laboratory conditions.

In the field survey, the first larvae were found at the beginning of July in the Bingöl region (1,000 m asl). The larvae fed on receptacle tissue enlarging a niche, eventually consuming all the flower parts and receptacle. Larvae fastened together remaining flower parts and frass to form a hard cell inside the flower head. Pupation occurs inside the flower head, about 3 days after mature larvae ceased feeding. Larvae were seen from the beginning of July to mid-September. Attacked flower heads can easily be distinguished because they dried prematurely without flowering.

First adult emergence was recorded in late July in Iğdır region. The adults waited inside flower heads about

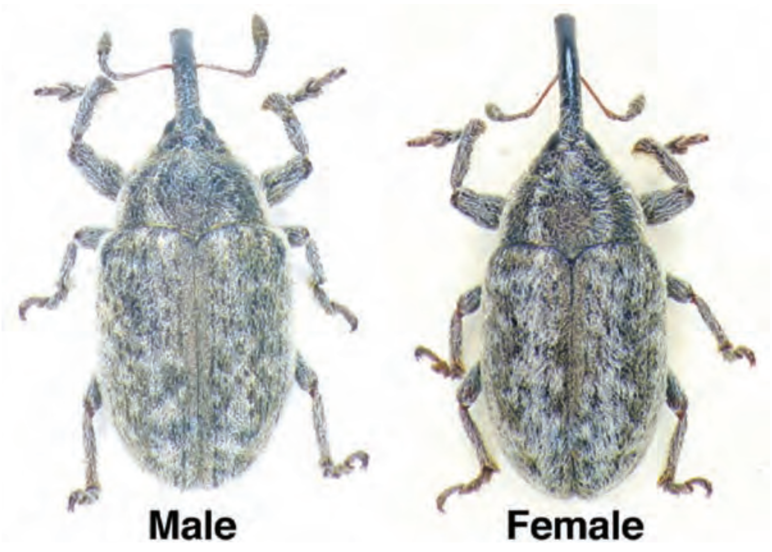


**Figure 1.** Adults of *Larinus filiformis* Petri.

1 week before exiting from the top of the flower. New adults were recorded in the field until mid-September at the Iğdır site. Hibernation started in mid- to late Sep tember: Adults generally hide under rocks, dry plant parts or debris in groups of two to three individuals.

## Geographical distribution and host plant range

*L. filiformis* occurs from eastern Armenia, Nakhichevan autonomous region of Azerbaijan and in Turkey, from the east border through central Anatolia and near the southern Mediterranean coast (Figure 2). One specimen was determined from Bulgaria, which was a new record for Europe (Gültekin *et al*., 2006).

Three years of extensive field observations, collections and laboratory rearing, carried out by our group in Turkey, indicate that *L. filiformis* is monophagous on *C. solstitialis*.

## Natural enemies

Six hymenopteran parasitoid species were reared from larval and pupal stages of *L. filiformis*: *Bracon urinator* (F.), *Bracon tshitsherini* Kok., *Exeristes roborator* F., *Aprostocetus* sp. and two unidentified wasp species belonging to the families Eurytomidae and Ormyridae.

# Conclusions

Our results indicate that *L. filiformis* is closely associated with *C. solstitialis* in the field in eastern Turkey. The insect was very common on *C. solstitialis* and was never found feeding on other nearby plants. The adults destroy many immature flower heads, and larvae destroy all the seeds within flower heads that they infest. This damage is very similar to that caused by *Larinus minutus* Gyll. on spotted and diffuse knapweeds, *Centaurea stoebe* L. and *Centaurea diffusa* L.; *L. minutus* Gyll. is a biological control agent that appears to be reducing populations of these two weeds in North America (Smith, 2004b; Seastedt *et al*., 2007). Therefore, *L. filiformis* warrants further evaluation as a prospective biological control agent for this weed, especially if it does not interfere with the other seed head insects that are already established (Pitcairn *et al*., 2004; 2005).

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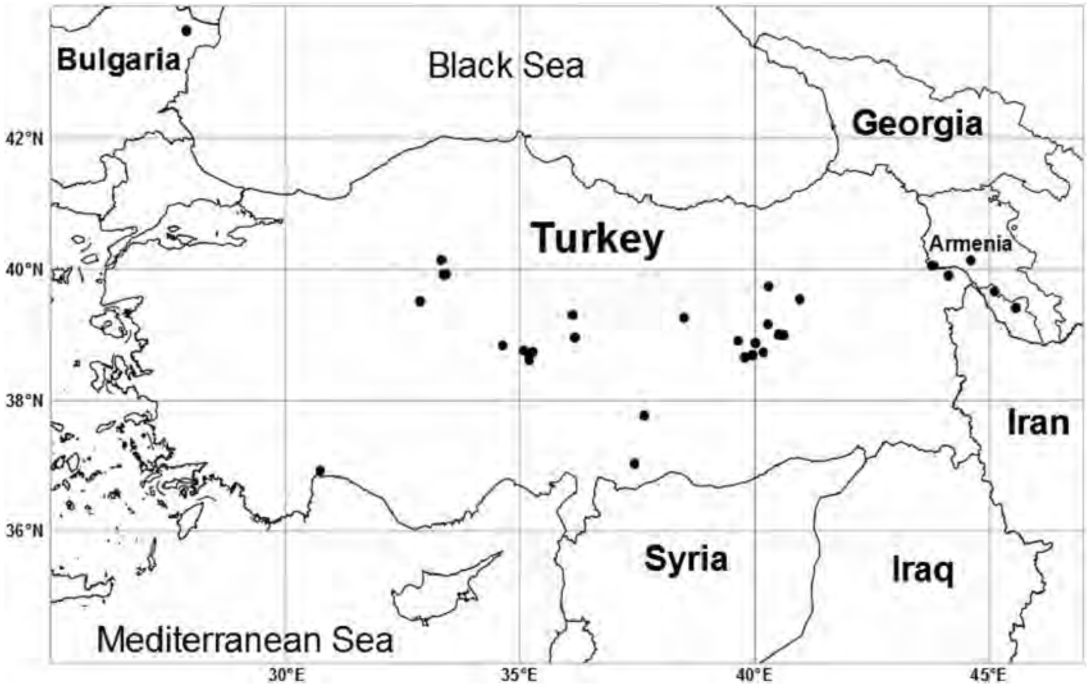


**Figure 2.** Geographic distribution of *Larinus filiformis*.

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# All against one: first results of a newly formed foreign exploration consortium for the biological control of perennial pepperweed

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*Summary*

Perennial pepperweed (PPW), *Lepidium latifolium* L., is a mustard of central Asian origin that is invading natural and cultivated habitats in North America and is difficult to control with conventional means. Biological control of PPW is hampered by the fact that it is relatively uncommon in its native range and that it has more than 30 native North American congeners. In addition, detailed information on phytophagous organisms and diseases associated with PPW in its native range is sparse. In 2005, a foreign exploration consortium was formed, and in 2006, five field trips were conducted: three to Turkey, one to Kazakhstan and one to Romania and Bulgaria. A total of 28 field sites of PPW were sampled. Based on identifications available thus far and combined with data of previous opportunistic surveys, we reared or sampled 67 phytophagous organisms, only seven of which have previously been recorded from PPW. Although plants in Kazakhstan showed more obvious signs of damage than in Turkey, a similar number of phytophagous organisms were collected. Only three species were found in both countries, indicating two distinct herbivore communities. At least six potential biological control agents were found during these first surveys: one root-mining weevil, *Melanobaris* sp. pr. *semistriata* Boheman, and an eriophyid mite, *Aculops* sp., in Turkey; one gall-forming weevil, *Ceutorhynchus marginellus* Schultze, a shoot-mining flea beetle, *Phyllotreta reitteri* Heikertinger, and a fungal leafspot pathogen, *Septoria lepidii* Desmazières, in Kazakhstan; and a shoot-mining chloropid fly, *Lasiosina deviata* Nartshuk, in both countries. The shoot-mining flea beetle in particular was found to be very damaging, causing the die-back of shoots. For *M.* sp. pr *semistriata* and *P. reitteri*, we have established a colony in quarantine at CABI and have started to investigate their biology.

***Keywords***: *Lepidium latifolium,* Turkey, Kazakhstan, field surveys.

## Introduction

Perennial pepperweed (PPW), *Lepidium latifolium* L. (syn.: *Cardaria latifolium*) (Brassicaceae), is a herbaceous, semi-woody perennial that typically reaches 0.5 to 1.5 m in height and reproduces vegetatively and by seed (Renz, 2000). Plants regrow early each year from a dense network of creeping, horizontal roots, flower in June/July and set seeds in July/August. PPW is a prolific seed producer, capable of producing more than six billion seeds per acre of infestation (Miller *et al.*, 1986).

PPW is native to central Asia and is believed to have been introduced into the United States through California around 1900 as a contaminant of sugar beet seeds (Renz and DiTomaso, 1998). It is now widespread across North America but especially prevalent in Nevada, Oregon, Utah and California. Perennial pepperweed is declared noxious or prohibited in 14 US states and Canadian provinces (US Department of Agriculture (USDA)–Natural Resources Conservation Service (NRCS) Plants National Database, http://plants.usda.gov; Invaders Database, http://invader.dbs.umt.edu).

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PPW is often associated with mesic habitats, such as riparian areas, drainage ditches and subirrigated pastures and hay meadows. However, it can invade a wide range of habitats including pastures, open fields, roadsides and residential areas (Young *et al.*, 1998). Downstream movement of seeds and root fragments along waterways and irrigation systems is the primary mode of spread for this weed. PPW is highly competitive, and invasions result in dense monocultures and subsequent loss of biodiversity through the exclusion of native, riparian vegetation (Trumbo, 1994; Young *et al.*, 1995; Blank and Young, 1997). In areas that are not mown annually, semi-woody stems can accumulate over the years, negatively impacting nesting habitat for birds and hindering the grazing and movement of livestock and wildlife (Renz, 2000). In addition, like *Tamarix*, PPW alters habitat by increasing soil salinity (Blank and Young, 1997), thereby hindering regeneration of native flora. Conventional control methods are not necessarily cost-effective or environmentally safe and must need to be repeated over several years to be successful. Therefore, CABI Europe-Switzerland (CABI E-CH), Biotechnology and Biological Control Agency (BBCA) and associated collaborators formed a foreign exploration consortium in 2005 to investigate the feasibility for biological control of PPW.

## Literature surveys

The native range and area of origin of PPW are not clearly understood because of high intraspecific variability and the fact that the plant has been cultivated as a spice and vegetable since the twelfth century (Hegi, 1986). The plant is probably native to central Asia, with a centre of species diversity in Tajikistan, Kazakhstan and western Mongolia (E. Jäger, personal communication).

During a literature search for phytophagous organisms associated with PPW, we found 23 insect species (Hinz *et al.*, 2007). Twelve of these are beetles, and nine are heteropterans. In addition, the list contains one mite and eight fungal pathogens. Overall, surprisingly little information is available on phytophagous organisms and diseases associated with PPW, especially compared to the closely related hoary cress, *Lepidium draba* L., another invasive perennial mustard, for which we found nearly 200 associated phytophagous organisms (Cripps *et al.*, 2006). Lack of detailed investigations on PPW in its area of origin may reflect its more eastern distribution and relative scarcity. This has been confirmed by results of our first field surveys that have already revealed new host associations with PPW (see below).

## Field surveys

In 2006, five field trips were conducted, three to central and northeastern Turkey, one to southeastern Kazakhstan and one to Bulgaria and Romania. During the three field trips to Turkey in April, June and October, ten PPW sites were found, while in Kazakhstan (14–31 May), 19 sites of PPW were found (Fig. 1). Literature records indicate that PPW occurs in northeastern Bulgaria and southern Romania; however, we were unable to find PPW at several locations mainly along the Danube River.

At each field site, adult insects were sampled, plants were dissected, and eggs and immature larvae were transferred into artificial diet for rearing. In addition, plant parts (leaves, shoots and roots) with immature stages or signs of mite or pathogen attack were collected and transported to the lab. Immature stages were reared to adult and sent, together with field-collected adults, to taxonomists for identification. Based on identifications available thus far and combined with data of opportunistic surveys during previous years (see Hinz and Cristofaro, 2005), we reared or sampled 65 species, 59 of which are probably associated with plants in the family Brassicaceae.

So far, 28 species have been identified from Kazakhstan and 25 from Turkey. Interestingly, only three insect species, based on identifications available thus far, were recorded from both countries, indicating the existence of distinct herbivore communities in both areas and endorsing the importance of conducting field surveys for PPW over a large geographic area. Similar to results of surveys on other brassica weeds (e.g. hoary cress), two thirds of insects sampled and reared are weevils (Curculionidae; $n = 30$) and leaf beetles (Chrysomelidae; $n = 16$). However, not all identifications have been completed.

### Kazakhstan

In terms of potential biological control agents, Kazakhstan is a very interesting country, presumably because it forms part of the area of origin of PPW. Sites were relatively easy to find but never very large. PPW growing along the roadside was usually relatively healthy or attacked by oligophagous or polyphagous insects, such as heteropterans. Plants with heavy insect and pathogen damage were mostly located in natural, relatively wet areas, such as the Ili River corridor, where PPW was often growing in close vicinity or intermixed with common reed, saltcedar and/or Russian olive.

Of particular interest are the flea beetle *Phyllotreta reitteri* Heikertinger, two weevil species *Lixus myagri* Olivier and *Ceutorhynchus marginellus* Schultze and the chloropid fly *Lasiosina deviata* Nartshuk (Table 1). Plants at several sites in Kazakhstan were attacked by a white rust, identified as *Albugo candida* (Pers.). The species is known to attack several crops in the mustard family and is already present in several areas in the western USA. Also identified were the pycnial and aecial stages of an unknown, possibly heteroecious rust, as well as the coelomycete fungus *Septoria lepidii* Desmazières, reported from several species within

**Figure 1.** Overview of field surveys conducted for potential biological control agents for *Lepidium latifolium*, perennial pepperweed, in 2006.

**Table 1.** Potential biological control agents for *Lepidium latifolium*, perennial pepperweed.

| Species | Family | Plant part attacked | Country found in |
|---|---|---|---|
| *Ceutorhynchus marginellus* | Curculionidae | Petioles, leaves, stems (gall inducer) | Kazakhstan |
| *Melanobaris* sp.n. *semistriata* | Curculionidae | Root, root crown | Turkey |
| *Phyllotreta reitteri* | Chrysomelidae | Stem | Kazakhstan |
| *Lasiosina deviata* | Chloropidae | Stem | Turkey, Kazakhstan |
| *Aculops* sp. | Eriophyidae | Inflorescences and leaves (?) | Turkey |
| *Septoria lepidii* | Coelomycete | leaf | Kazakhstan |

the genus *Lepidium* (*Cardaria*). Identification of other disease symptoms is still under way. Small flea beetles occurring in large numbers on PPW were also collected. However, most of these were identified as oligophagous or polyphagous species. In addition, two weevil larvae were found mining in the root crown of PPW at one site, and leaf-mining flies were collected at several sites. Unfortunately, none of these could be reared through to adult.

## Turkey

We concentrated our surveys in eastern Turkey and in Central Anatolia and the Ankara region. As in Kazakhstan, most sites were in relatively wet areas, i.e. along rivers, irrigation channels and sewage ditches, in both natural and urban areas.

Compared to Kazakhstan, plants in Turkey showed less obvious signs of damage. Nevertheless, a similar number of phytophagous organisms was collected (see above). Three potential biological control agents were found: the weevil *M.* sp. near *semistriata* Boheman, the chloropid fly *L. deviata* and an eryophyid mite, preliminarily identified as *Aculops* sp. near *lepidii*. Two additional species of potential interest are an as yet unidentified flea beetle, *Psylliodes* sp., that probably mines in the shoots of PPW, and the root-mining weevil *L. myagri*. However, the latter is reported to be oligophagous (Dieckmann, 1983).

# Potential biological control agents

## *Melanobaris*. sp. pr. *semistriata* (Coleoptera: Curculionidae)

This root-mining weevil is recorded from several PPW sites in Turkey, and data on its biology and phenology have been collected since 2002 (Gültekin *et al.*, unpublished data). Adults usually become active in mid-May and feed on young petioles and the base of stems. In mid-June, the first larvae were found feeding in the petioles. Larvae then move down to the root crown, where they also pupate. New generation adults emerge in the autumn. During the field trip to Turkey at the beginning of April 2006, 28 adults were collected and taken back to Switzerland to establish a colony in quarantine and develop methods for host-specificity tests.

Weevils were either released onto potted plants of PPW or transferred into cylinders and offered cut plant material inserted in wet florist foam blocks. Oviposition occurred in the foam material, which may suggest that in the field, *M.* sp. pr. *semistriata* females lay eggs, not as most other weevil species into the plant tissue but into the soil close to the root crown. Host-specificity tests will start in 2007.

The weevil is very close to *M. semistriata* Boheman, normally developing on *L. draba* (hoary cress), another invasive Brassicaceae studied at CABI, but may well represent a distinct undescribed species (Korotyaev, unpublished data).

## *Ceutorhynchus marginellus* (Coleoptera: Curculionidae)

During the field trip to Kazakhstan in May 2006, we found several sites, at which PPW plants were attacked by this gall-inducing weevil. Not much information is available on the species, except that it has been collected previously on PPW in Russia (Isaev, 1994) and also occurs in Kazakhstan. Galls are formed on leaf midribs, leaf stalks and stems, and weevil larvae were mining inside. Gall induction appears to stunt shoot growth. At one site, we also found adults of *C. marginellus*. Infested material and adult weevils were returned to CABI's quarantine facility in Switzerland. Mature larvae left the material to pupate in the soil, and adults emerged between 12 and 27 June. Weevils were artificially overwintered in an incubator at 3°C and started to lay eggs in March 2007.

## *Phyllotreta reitteri* (Coleoptera: Chrysomelidae)

At two field sites in Kazakhstan in May, we found PPW plants that were heavily attacked by larvae of *P. reitteri*. The species is also recorded from Uzbekistan and the Crimea Peninsula (Lopatin, 1977). No information is available on the biology of this species. We observed larvae mining in the stems of PPW. At one site, nearly 100% of plants were attacked, and heavy attack caused the die-back of shoots. We took infested material back to CABI's quarantine facility in Switzerland, and between 16 June and 7 July, adults emerged. Beetles were successfully overwintered and started to

lay eggs in March 2007. We are currently transferring larvae onto potted PPW plants to establish a colony and conduct preliminary host specificity tests.

### *Lasiosina deviata* (Diptera: Chloropidae)

This chloropid stem-mining fly was reared for the first time from PPW in June 2005 (Gültekin, unpublished data) and later described by Nartshuk (2006). During surveys in 2006, we mainly found it in central Turkey but presumably also at one field site in Kazakhstan. However, whether the flies from Kazakhstan are exactly the same species as the ones collected in Turkey still needs to be confirmed. The fly can be quite frequent; for example, at one site 60% of shoots were attacked, and up to 11 larvae were found mining in one stem (mean ± SE: 3.8 ± 1.1). In June, mature larvae and pupae were found. Unfortunately, most adults emerged during the field trip and died, so no further studies could be initiated.

The genus *Lasiosina* comprises 34 species in the Palaearctic (Nartshuk, 2006). This is the first record of a *Lasiosina* species from a dicotyledon plant. The flies usually develop on cereals and grasses (Poaceae). We are therefore planning to check monocot plants growing intermixed with PPW at *L. deviata* field sites for attack and conduct preliminary host-specificity tests concentrating on one or two important cereals (e.g. wheat) and grass species. Depending on results, we will make a decision whether *L. deviata* can truly be regarded as a potential biocontrol agent for PPW.

### *Aculops* sp. (Aceria: Eriophyidae)

This eriophyid mite was only found at one field site in Central Turkey in June 2006. Plants attacked by the mites appeared to have stunted inflorescences and showed discoloration. The species resembles *A. lepidii* Roivainen, which was originally described from a single location within the Sierra Nevada, Spain (Roivainen, 1953). It was only found associated with *Lepidium stylatum* (Lag. et Rodr.) and is reported to attack leaves, causing abnormal pilosity, leaf margin rolling and curling. The differences in symptoms, host plant and habitat conditions between *A. lepidii* and the mite found in Turkey indicate that it is most probably a new species or subspecies. During a field trip in April to Turkey, we found PPW plants with curled leaves, symptoms resembling those described for *A. lepidii*. More material will be collected and sent to taxonomists in 2007 to resolve the taxonomic position of the mites found and to make a complete morphological description.

### *Septoria lepidii* (Mycosphaerellaceae: Dothideales)

The coelomycete *Septoria lepdii* was also only found at one PPW site in Kazakhstan, causing leafspots on infected host plants. Although it is reported from a number of *Lepidium* species in Europe and Asia, specific host strains might exist. *Septoria* species, constituting the asexual stage of *Mycosphaerella* sp., have previously been used successfully as biological control agents, i.e. *Septoria passiflora* Syd. against the alien invasive *Passiflora tripartita* (Juss.) Poir. (Banana Poka) in Hawaii (Julien and Griffiths, 1998). We are planning to collect more material in 2007 and start preliminary pathogenicity and host-specificity tests.

## Conclusions and outlook

Results of these first field surveys are very promising and have revealed several insects and one fungal pathogen with potential for biological control of perennial pepperweed. We have already established colonies of two species, one root-mining weevil and one stem-mining flea beetle and started preliminary host-specificity tests. In 2007, we will collect material of at least two additional species. The identification of all remaining material collected in 2006 will be concluded, and depending on results, a revised list of potential agents prepared. In addition, we will continue and extend field surveys to regions not yet covered, such as western China, Mongolia, southern Russia, and southern Ukraine. Finally, we will collect herbarium specimens and material for molecular analysis to better understand the genetic variability of PPW in Eurasia and the origin of invasive populations in North America.

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# Potential biological control agents for fumitory (*Fumaria* spp.) in Australia

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*Summary*

*Fumaria* species are increasingly problematic in the cropping regions of southern Australia, and one fumitory, *Fumaria densiflora* DC, has developed populations with herbicide resistance. Consequently, the potential for biological control was assessed. Nine species of fungi were found associated with *Fumaria* spp. in a survey of 33 sites in southern France. According to the literature, species potentially host specific to fumitory include *Cladosporium brachormium* Berk. and Broome, *Entyloma fumariae* J. Schröt. and *Peronospora affinis* Rossmann. Of the insects detected on *Fumaria* spp. in France, the stem weevil, *Sirocalodes mixtus* Mulsant and Rey has potential as a biological control agent because it is thought to be host specific. None of these species were detected amongst the six pathogen species found during surveys of 64 locations in southeastern and southwestern Australia. The absence of pathogens and insects associated with *Fumaria* species in Australia, the lack of *Fumaria* spp. native to Australia, and few closely related crops or ornamental species, indicate that there are opportunities for research into the development of natural enemies for the biological control of fumitory.

***Keywords***: *Fumaria* species, biological control, field surveys, fungal pathogens, arthropods.

## Introduction

Fumitory species are weeds of many parts of the world, mainly in cereal and legume cultivation, vineyards, wastelands and gardens, but have not been considered as a target for biological control. In Australia, fumitory is a problem in canola and pulse crops (particularly peas and lupins; Holding and Bowcher, 2004). Lemerle *et al.* (1996) found *Fumaria* species in 37% of 86 cereal crops in southern New South Wales during a survey of weeds conducted in spring 1993. Agronomists and farmers from this region ranked fumitory in the top ten of 50 species in terms of potential threat. *Fumaria densiflora* DC first evolved resistance to group K1/3 herbicides (Ditroanilines and others) in New South Wales (NSW) (Heap, 2007), underlining that this species is the most important weed of the genus in Australia (Norton *et al.*, 2004). In Australia, all *Fumaria* species (seven species and two subspecies: *Fumaria bastardii* Boreau, *Fumaria capreolata* L., *Fumaria capreolata* L. subsp. *capreolata*, *F. densiflora*, *Fumaria indica* (Hausskn.) Pugsley, *Fumaria muralis* Sond. ex W.D.J.Koch, *Fumaria muralis* Sond. ex W.D.J.Koch subsp. *muralis*, *Fumaria officinalis* L., *Fumaria parviflora* Lamarck) are introduced, mostly from Europe (Australian National Botanical Garden, 2007 a, b; Norton, 2003). *Fumaria* species were found in all States of Australia and are abundant only in southern regions, mostly in New South Wales, Victoria, South Australia and Western Australia. The increasing importance of *Fumaria* spp. and the rise of herbicide resistance point to the need to investigate alternative means of control. This paper reports on preliminary surveys in France and Australia to find potential biological control agents.

## Methods

### Surveys in France

Surveys were conducted in France from April 2004 to May 2005. Populations of *Fumaria* were found at 33 sites out of 79 inspected, either in vegetable culture or vineyards or on areas of freshly disturbed soil. The sites were distributed along five transects covering the Riviera between Hyères, St Tropez and Lake Ste Croix

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(circa 270 km), the Cévennes between Mount Aigoual and Montpellier (ca. 110 km), the Pyrénées between The Boulou and Prades (ca. 130 km), the Atlantic coast between Tarbes and St Jean de Luz (ca. 200 km) and Bordeaux to Agen (ca. 150 km) and one longer transect through agricultural regions (ca. 1580 km).

At each site, plants were searched visually for evidence of disease. Samples of diseased tissue were collected and examined in the laboratory. When a pathogen was found associated with a symptom, after its fructification in a humid chamber, a single spore was collected with a sterile needle under a stereomicroscope, deposited on a potato dextrose agar (PDA; Difco) Petri dish and placed in a controlled environment (20°C and 12 h photoperiod). Fungi were identified morphologically by microscopic observations.

Plants were searched for evidence of insects at each site, and from four to ten entire plants were collected then taken back to the laboratory in transparent plastic bags for dissection. Plants with evidence of insect damage were cut up and put in Petri dishes, on wet paper, to allow eggs or larvae to develop to the adult.

## Surveys in Australia

Transects were made through grain-growing regions in southeastern Australia (NSW and Victoria) and southwestern Australia between April and October 2005. The transects were made through districts where *Fumaria* species have been previously recorded, and crops and other roadside habitats were briefly searched for the presence of fumitory at intervals of approximately 25 km along the length of each transect. The transect distance travelled was approximately 1200 km in each of NSW and Victoria and 1800 km in southwestern Australia. At sites where fumitory was detected, at least 20 plants from that site were examined closely over a 20- to 30-min period for symptoms of disease and insect attack. Where arthropods were detected or signs of arthropod attack were observed, this was noted. When fruits were present, these were examined from at least 20 plants using a 10  hand lens for evidence of insect-caused galls.

Representative samples from each disease category were collected from each site, placed into polyethylene bags and stored in an insulated cool box with ice until they were brought to the laboratory. Leaf symptoms were recorded, and pathogens were isolated and identified as described below.

**Fungal isolation and species identification:** Subsamples of diseased material were placed into humid chambers for up to 14 days at 15°C and 20°C under a 12 h near-UV light: 12 h dark photoperiod to encourage fungal sporulation. The humid chambers consisted of two to three layers of moistened sterile filter paper in sterile 90 mm Petri dishes or plastic 'take-away' containers (150 mm  80 mm  50 mm) sealed with Parafilm wrap or plastic cling film (Gladwrap). In addition, diseased shoot and root material was surface disinfested by soaking in 2.5% sodium hypochlorite (*v*/*v*) for 1 min, rinsed in three changes of sterile tap water, blotted dry and plated onto 2% malt extract agar (MEA; Difco) or water agar (20 g Difco agar made up to 1 l with deionised water). Pure fungal cultures were obtained by transferring single spores or single hyphal tips onto fresh media and maintained on MEA, PDA or V8 juice agar (200 ml Campbell's V8 juice, 3 g CaCO3, 15 g Difco agar made up to 1 l with deionised water). Cultures were grown under white fluorescent and near-UV light in a 12 h light:12 h dark photoperiod at 20°C in order to induce fruiting structures to facilitate species identification.

Small pieces of culture were mounted under acidified glycerol blue [0.05 % aniline blue (Gurr) in lacto glycerol] and investigated under the light microscope for the formation of fructifications. Fungal structures were investigated on normal or phase-contrast settings (100–1000  ), and 30 measurements were made of conidial dimensions. Fungi were identified according to standard mycological texts, and original descriptions where indicated.

# Results

Twelve genera including 15 identified species of fungal pathogens have been reported in the literature as associated with *Fumaria* spp. (results not shown). Three species are considered to be potentially host specific at the level of the plant family and genus (*Cladosporium brachormium* Berk. and Broome (anamorphic Mycosphaerellaceae), *Entyloma fumariae* J. Schrot. (Entylomataceae) and *Peronospora affinis* Rossmann (Peronosporaceae)).

## Surveys in France

**Pathogens:** Nine species of fungi were found associated with *Fumaria* species. Diseased leaves of *Fumaria* sp. with symptoms of leaf smut, *E. fumariae*, were collected from six sites in November, January and April. Symptoms of leaf smut appear as whitish spots on both sides of *Fumaria* leaves and occasionally petioles. Lesions become dark brown and entire leaves shrivel and eventually die. The three kinds of spores were observed, two external: primary sporidia (filiformes) and secondary sporidia on sterigma (ballistospores). Microscopic examination of internal leaf tissues revealed masses of round, double-walled, pale green-to-yellow spores typical of the ustilospores of *Entyloma*. *E. fumariae* was grown on artificial culture medium (PDA) and formed ballistospores that

have a similar structure and form to those produced on plants.

*P. affinis* was found on *Fumaria* spp. in ten sites of the 33 sites surveyed in France. The first symptoms observed were small faint yellow spots on the upper foliage. As they matured, fungus fruiting structures were visible on the lower leaf surface as conidiophores bearing conidia. Two kinds of spores were observed in field material: conidia on conidiophores and oospores in infected leaves.

Diseased plants of *Fumaria* spp. with symptoms of a *Cladosporium* species were collected from 13 sites. Extensive sporulation on leaves, inflorescences and stems were observed.

Two *Alternaria* spp. (anamorphic Pleosporaceae), a *Botrytis* sp. (anamorphic Sclerotiniaceae), a *Colletotrichum* sp. (anamorphic Phyllachoraceae), an *Oidium* sp. (anamorphic Erysiphaceae) and *Phomopsis leptostromiformis* (J.G. Kühn) Bubák (Valsaceae) were also found associated with diseased plants of *Fumaria* spp.

**Arthropods:** The only two polyphagous aphid species cited in the literature on *Fumaria* spp. were observed at three sites in France, *Aphis fabae* Scopoli and *Macrosiphum euphorbiae* (Thomas). A polyphagous lepidoptera, *Plusia* sp. (Noctuidae), was also observed at five sites. Otherwise, *Sirocalodes mixtus* Mulsant and Rey (Curculionidae), with stem-mining larvae, was the most common insect, being found at 13 of 33 sites.

## Surveys in Australia

**Pathogens:** The survey found fumitory at 64 of the 160 sites visited throughout the grain-growing districts of southern Australia. Six fungal species were identified from diseased fumitory plants: *Alternaria alternata* (Fr.) Keissl. (anamorphic Pleosporaceae), *Alternaria dauci* (J.G. Kühn) J.W. Groves and Skolko (anamorphic Pleosporaceae), *Davidiella tassiana* (De Not.) Crous and U. Braun (Mycosphaerellaceae), *Phoma* sp. (anamorphic Pleosporaceae), *Pleospora* sp. (Pleosporaceae) and *Sclerotinia* sp. (Sclerotiniaceae). In addition, several saprophytic and unidentified fungi that failed to sporulate were isolated from diseased material (data not shown). *Olpidium brassicae* (Woronin) P.A. Dang. (Olpidiaceae), previously recorded on fumitory in Western Australia (WA), was not recorded in the current survey. No pathogenic bacteria or nematodes were detected on fumitory.

The fungi were identified from six different fumitory species. *F. muralis* and *F. capreolata* were the only two species present in the WA surveys. In addition to these two species, *F. bastardii*, *F. densiflora*, *F. officinalis* and *F. parviflora* were observed in the surveys of eastern Australia. The plants were not identified to subspecies rank. *F. indica* was not detected in the current survey.

**Arthropods:** No arthropods were found on *Fumaria* in Australia during the surveys. There was evidence of possible sap-feeding insects on some plants at a few sites; however, no causative insects were present on the plants. No arthropods associated with fumitory have been reported from Australia in the literature.

# Discussion

The genus *Fumaria* is placed in the Subfamily Fumarioideae in the Family Papaveraceae (Lidén, 1986; Stevens, 2007). In earlier taxonomic treatments, the subfamily is treated as the Family, Fumariaceae. *Fumaria* is chiefly a Mediterranean group of 55 species with few species reaching India and east Africa (Lidén, 1986). There are no native Australian species in the Family Papaveraceae; the only genera of this family in Australia apart from *Fumaria* are introduced species of *Argemone* (three species), *Dicentra* (one species), *Eschsholzia* (one species), *Glaucium* (two species), *Hypecoum* (one species), *Papaver* (six species), *Platycapnos* (one species), *Pseudofumaria* (one species), *Roemeria* (one species) and *Romneya* (two species) (Australian National Botanical Gardens, 2007a,b). The only closely related crop to *Fumaria* is *Papaver somniferum* L. (poppy) which is grown in Tasmania for production of pharmaceutical chemicals (Laughlin *et al.*, 1998). *Fumaria* species are also weeds in this crop (Baldwin, 1977). Related ornamentals grown in Australia include species of *Chelidonium*, *Corydalis*, *Dicentra*, *Eschscholzia*, *Glaucium*, *Macleaya*, *Meconopsis*, *Romneya* and *Pseudofumaria* (Spencer, 1997). Thus the absence of related native species and few related commercial species indicates that weedy *Fumaria* species could be ideal targets for biological control.

## Surveys

The identification of pathogens is provisionary, and further work is needed to confirm identifications with molecular techniques or by appropriate experts. The sampling was carried out in France during an exceptionally dry year (2005). Even so, up to five pathogens were found per site, and what appeared to be sites with damaging infestations were found for *P. affinis*, *E. fumariae* and *P. leptostromiformis*. A more complete picture of the number of pathogens could be obtained by extending the survey to other western and southern European areas where there is an abundance of *Fumaria* species (Tutin *et al.* 1993). However, based on the literature, the survey in France has found most organisms of interest. This is also the first report of presence of *E. fumariae* in France. The sampling in Australia shows that this fungus and the other potential agents are highly likely to be absent.

## Pathogens

*E. fumariae* appears to be the most promising potential agent especially as it attacks several *Fumaria* species and is not present in Australia. *E. fumariae* has been found on *F. muralis* in Madeira Islands, *Fumaria rostellata* Knaf in Romania and *Fumaria vaillantii* Loisel. in Germany and Sweden (Vanky, 1994) and on *F. parviflora* in India (Zundel, 1953). Vanky (1994) noted about *E. fumariae* in Europe 'Rarely reported, almost certainly because *E. fumariae* is very inconspicuous'.

Despite this, recent experience shows that *Entyloma* species can be successful agents. Barton *et al.* (2007) report that *Entyloma ageratinae* R.W. Barreto and H.C. Evans was introduced in 1998 with success for the biological control of mist flower, *Ageratina riparia* (Regel) R.M. King and H.Robinson, in New Zealand. By 2004, the proportion of leaves infected by the fungus increased to 60%. The estimated cover of *A. riparia* in heavily infested plots decreased from 81% to 1.5% with a corresponding recovery of the diversity of native vegetation (Barton *et al.*, 2007).

*P. affinis*, an obligate plant parasite, was abundant at three sites in France and not reported from Australia. It is associated with *Fumaria* species making it a candidate for further consideration for biological control. However, there is a report in Farr *et al.* (2007) of a 1902 herbarium specimen from Italy with *P. affinis* on the leaves of *Chenopodium album* L. (Chenopodiaceae). Host range tests are needed to establish if this is a valid host.

The third species that could be considered is *C. brachormium* since it is only known from *F. officinalis*. It has not been reported from Australia. *Cladosporium* species are known generally as saprophytes, but they can be also pathogens. Otherwise, there appears to be very little known about this fungus.

## Insects

Few insect species were found during the surveys and most of them are polyphagous. Little information on fumitory and insects is available in the literature. Three curculionids are cited on *Fumaria* (Hoffmann, 1954). *Sirocalodes quercicola* Paykull is a gall former developing in crown of *F. officinalis* and *F. capreolata*. This species is found in Europe but is not common. *Sirocalodes nigrinus* Marsham is common in France particularly in the southeast coastal region. The larvae develop in stems on *F. officinalis* and *F. parviflora*. The adult was also recorded on *F. vaillantii*, *F. capreolata, Platycapnos spicata* (L.) Bernh. (Hoffmann, 1954) and *Papaver rhoeas* L. (Campobasso *et al.*, 1999).

During our surveys, we only found the third species, *S. mixtus*. It occurs throughout France, but it is more common in the Mediterranean region (Hoffmann, 1954). This species could be considered as a potential agent, and further studies are needed to define the host-plant specificity of this insect. It has only been associated with *F. officinalis* and *F. parviflora* in France (Hoffmann, 1954). Adults have been found on *Papaver hybridum* L. in Portugal (Campobasso *et al.*, 1999).

A second arthropod that is a potential agent is the cynipid wasp, *Neaylax versicolor* (Nieves-Aldrey). The larvae of this wasp form galls in the fruits of *Fumaria* species. It is the only cynipid found in *Fumaria* spp. (Nieves-Aldrey, 2003; Askew and Nieves-Aldrey, 2005) and has a distribution including southern France, Greece and Spain. We did not find it in our survey as most of the plants were just flowering, and the few green fruits did not show any obvious wasp damage.

# Conclusions

We have identified suitable organisms to be considered for a biological control project in Australia. Any future project will require relevant authorizations, and this will be helped by a clearer understanding of the taxonomy, identification and importance of *Fumaria* species from Australia and the cost–benefits of control in cropping systems.

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# Expanding classical biological control of weeds with pathogens in India: the way forward

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*Summary*

Invasive alien weeds are a major constraint in agriculture, forestry and the environment in India. Classical biological control (CBC) of these exotic weeds through deliberate introduction of arthropods is almost a century-old practice. History has recently been made with the successful introduction of the first plant pathogen, *Puccinia spegazzinii* de Toni, against mikania weed (*Mikania micrantha* H.B.K.) in India. With the mechanism in place for the importation, quarantining and release of pathogens, it is envisaged that more introductions will be made in the future. The agent under immediate consideration is *Puccinia abrupta* Diet. and Holw. var. *partheniicola* (Jackson) Parmelee against parthenium weed (*Parthenium hysterophorus* L.), generally considered as the worst terrestrial social weed in India. Down the line, other terrestrial weeds such as *Chromolaena odorata* (L.) R. King and H. Robinson and *Lantana camara* L. and aquatic species like *Eichhornia crassipes* (Martius) Solms-Laubach could be targeted for pathogens. This article, besides presenting an overview of the research that has gone into selection of candidate fungi for CBC of *M. micrantha* and *P. hysterophorus*, also analyses the infrastructure and expertise requirements for further expanding the target list.

***Keywords***: *Mikania micrantha*, *Parthenium hysterophorus*, invasive alien weeds, Indian infrastructure for biological control, rust pathogens.

## Introduction

The impact of invasive alien weeds on agriculture, horticulture, forestry and the environment has been felt for centuries in India. History is replete with examples of reports and records of 'new' and 'emerging' or 'invading' weeds. In India, traditionally an aggressive trading nation, movement of unwanted plants into and out of the country was probably widespread before the government-run quarantine system came into existence with the promulgation of the Destructive Insects and Pests Act in 1914.

Classical biological control (CBC) of these exotic weeds through deliberate introduction of natural enemies, principally arthropods, has been in practice for almost a century in India. Surprisingly, however, pathogens have not received much attention in India though the approach of manipulating plant pathogens for suppressing troublesome weeds has been known to science for more than a century (Wilson, 1969).

Nevertheless, India has now caught up with the rest of the pioneers in the field by recently introducing a host-specific plant pathogen, *Puccinia spegazzinii* de Toni, against mikania weed (*Mikania micrantha* H.B.K.), and thereby became the eighth country in the world to practise CBC of weeds with plant pathogens (Kumar *et al.*, 2005; Ellison *et al.*, 2006). It took more than three decades for India to adopt this strategy since the first successful use of an introduced pathogen elsewhere in the world, i.e. control of the skeleton weed, *Chondrilla juncea* L., in south-east Australia with the rust fungus, *Puccinia chondrillina* Bubak and Sydenham, was successfully implemented in the early 1970s (Cullen *et al.*, 1973).

## A brief history of CBC of weeds in India

India has traditionally been one of the early-adopters of CBC of insect pests and weeds alike (see Table 1).

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**Table 1.** Exotic natural enemies field-released for CBC of weeds in India.

| Weed (purported year of introduction) | Agents released (year)[a] | Establishment in the field and impact |
|---|---|---|
| **Terrestrial weeds** | | |
| *Ageratina adenophora* (Sprengel) R. King and H. Robinson (1900) | *Procecidochares utilis* Stone (1963) | Established - minimal control due to parasitoids |
| *Chromolaena odorata* (L.) King and H. Robinson (1914) | *Apion brunneonigrum* Béguin Billecocq (1972) | Not established |
| | *Pareuchaetes pseudoinsulata* Rego Barros (1973 and 1984) | Recently reappeared |
| | *Cecidochares connexa* (Macquart) (2005) | Established - too early to assess |
| *Lantana camara* L.(1809) | *Ophiomyia lantanae* (Froggatt) (1921) | Established - not effective |
| | *Teleonemia scrupulosa* Stål (1941) | Established - provides minimal control |
| | *Diastema tigris* Guenée (1971) | Not established |
| | *Salbia haemorrhoidalis* Guenée (1971) | Not established |
| | *Octotoma scabripennis* Guérin-Méneville (1972) | Established - not effective |
| | *Uroplata girardi* Pic (1972) | Established - not effective |
| *Mikania micrantha* H.B.K (1914) | *Puccinia spegazzinii* de Toni (rust pathogen, 2005 Assam and 2006 Kerala) | Established in Kerala - too early |
| *Opuntia* spp. (unknown) | *Dactylopius ceylonicus* (Green) against *Opuntia vulgaris* Miller (1795) | Established and provided excellent control |
| | *Dactylopius confusus* (Cockerell) against *O. vulgaris* (1836) | Not established |
| | *Dactylopius opuntiae* (Cockerell) against *Opuntia elatior* Miller and *Opuntia stricta* (Haworth) Haworth var. *dillenii* (Ker Gawler) L. Benson (1926) | Established and provided complete control of both species |
| *Parthenium hysterophorus* L. (1955) | *Zygogramma bicolorata* Pallister (1984) | Excellent control in some areas |
| **Aquatic weeds** | | |
| *Eichhornia crassipes* (Martius) Solms-Laubach (1900) | *Neochetina eichhorniae* Warner (1983) | Established - provides good to variable control |
| | *Neochetina bruchi* Hustache (1984) | Established - provides good to variable control |
| | *Orthogalumna terebrantis* Wallwork (1986) | Established - alone not very effective |
| *Salvinia molesta* Mitchell (1955) | *Paulinia acuminata* (Degeer) (1974) | Established - uncertain control |
| | *Cyrtobagous salviniae* Calder and Sands (1983) | Established - spectacular control |

[a]All agents are arthropods except where indicated otherwise.

Although the first exceptional success in CBC of a weed was in fact achieved with an erroneous introduction in 1795, that laid the foundation for further imports of specific natural enemies as a result of the realization of the potential of the approach. The agent in question was the mealybug *Dactylopius ceylonicus* (Green) introduced from Brazil in place of *Dactylopius coccus* Costa, the species intended for commercial production of cochineal dye. *D. ceylonicus* dramatically brought down the population of the prickly pear cactus, *Opuntia vulgaris* Miller, within 5 to 6 years in central and north India (Singh, 1989).

The same episode gave a lesson on the significance of host specificity as well. *D. ceylonicus*, when tried against *Opuntia stricta* (Haworth) Haworth var. *dillenii* (Ker Gawler) L. Benson [=*Opuntia dillenii* (Ker Gawler) Haworth], could not suppress the weed in south India. Subsequently, the intentional introduction of a North American species, *Dactylopius opuntiae* (Cockerell), from Sri Lanka in 1926 into India resulted in impressive control of *O. stricta* and the related *Opuntia elatior* Miller. This was the first successful intentional use of an insect to control a weed in India, and more than 40,000 ha area was thus cleared (Singh, 1989; Julien and Griffths, 1998).

The opuntia experience resulted in a series of introductions of phytophagous insects such as *Ophiomyia lantanae* (Froggatt) (ex Mexico, via Hawaii in 1921) against lantana weed (*Lantana camara* L.) *Procecidochares utilis* Stone (ex Mexico, via New Zealand in 1963) against crofton weed [*Ageratina adenophora* (Sprengel) R. King and H. Robinson] and *Pareuchaetes pseudoinsulata* Rego Barros (ex Trinidad in 1973) against Siam weed [*Chromolaena odorata* (L.) King and H. Robinson] (Julien and Griffths, 1998).

In the post-independence era, CBC became more systematic and scientific with specific programmes managed by the erstwhile Indian Station of the Com-

monwealth Institute of Biological Control (CIBC) based in Bangalore. Later, country-specific programmes came under the purview of the All-India Coordinated Research Project (AICRP) on Biological Control of Crop Pests and Weeds, which was launched in 1977. This programme eventually came under the auspices of the Project Directorate of Biological Control (PDBC), which was formed in October 1993 under the Indian Council of Agricultural Research (ICAR). For weed control, only an insect and a plant pathogen have been introduced since the formation of PDBC. Table 1 provides the list of natural enemies other than fish imported for biological control of weeds in India and the current status of their impact.

## An overview of two pathogen-based weed CBC projects in India

### Target weed 1: *Parthenium hysterophorus*

The worst terrestrial social weed in India, *Parthenium hysterophorus* L., in general referred to as parthenium weed or ‘congress grass’, has been the primary target for possible biological control using both insects and pathogens.

Under the UK Department for International Development (DFID)-sponsored collaborative project between CABI Europe-UK (formerly CABI Bioscience) and the ICAR, between 1996 and 2000, development of both classical and the bioherbicide approaches were given prominence. Research in India culminated in the identification of a range of fungal pathogens of parthenium weed in Karnataka and Tamil Nadu in the south, Madhya Pradesh, Haryana, Punjab, Himachal Pradesh, Delhi and Uttar Pradesh in the north (Evans *et al.*, 2000). A few of these pathogens, despite possessing some potential as mycoherbicides, did not warrant the significant costs involved in further product development (Kumar and Evans, 2005).

In parallel research, two rust species were considered as options to be CBC agents, *Puccinia melampodii* Diet. and Holw. and *Puccinia abrupta* Diet. and Holw. var. *partheniicola* (Jackson) Parmelee. Both of these damaging rusts originate from Mexico and have already been fully screened and released in Australia for the control of parthenium weed. A comparison between these two rust species is given in Table 2.

*P. abrupta* var. *partheniicola* was found to severely reduce both the vegetative growth of young plants and the seed production of older plants under glasshouses conditions (Evans, 1987a, b). This rust is also known to be present in its exotic range, including India, though the strains do not appear to be widespread or aggressive as they are in their native range (Kumar and Evans, 2005). In India, the rust was first reported from an elevated site (930 m) (Evans and Ellison, 1987, cited by Parker *et al.*, 1994).

Unconfirmed reports suggest that *P. abrupta* var. *partheniicola* also occurs at lower elevations in India, but it is not a common pathogen of the weed (Kumar and Evans, 2005). A Mexican isolate (CABI no. W1905) of this rust was, however, found to be virulent and damaging to 12 *P. hysterophorus* collections from across India (Evans *et al.*, 2000).

Mexican isolates of *P. melampodii* (CABI nos. W1496 and W1500) were also found to be highly virulent towards the 12 Indian collections of *P. hysterophorus* producing high infection level and sporulation (Evans *et al.*, 2000). This rust was considered for introduction in India under the DFID project. However, the ability of the rust to infect calendula (*Calendula officinalis* L.) under glasshouse conditions could not be tolerated in India (as it was in Australia). Thus, field-based host-range testing was undertaken in Australia to see if Indian varieties of calendula could be infected under ‘natural’ conditions. Unfortunately, they were susceptible, and consequently the rust was not released in India.

### Target weed 2: *Mikania micrantha*

The neotropical vine mikania weed is an increasing threat to natural and man-made forests as well as to several agricultural and horticultural ecosystems in India. Although in its native range *M. micrantha* is rarely weedy, in its exotic range, especially in south and south-east Asian countries, it has become an intractable weed over the past several decades. Because of its rapid growth habit, the plant, which can smother even such hardy trees as teak, eucalyptus, rubber, oil palm

**Table 2.** A comparison between two rust species used for the control of parthenium weed.

| *Puccinia melampodii* | *Puccinia abrupta* var. *partheniicola* |
|---|---|
| Microcyclic, autoecious rust-producing telia and basidiospores | Macrocyclic, autoecious rust-producing uredinia and telia in the field. Pycnia and aecia have been induced in glasshouse conditions (Evans, 1987b) |
| ‘Summer rust’ - found in the humid and warmer lowland plains of the Caribbean coast of Mexico (Evans, 1997) | ‘Winter rust’ - found predominantly in the semi-arid, uplands of northern Mexico |
| Not present in India | Strain found in India (Evans and Ellison, 1987, cited in Parker *et al.*, 1994) |
| Infects calendula | Highly host-specific |

and cocoa, has acquired one of its common names, mile-a-minute weed. Whereas in north-east India it is a great problem particularly in tea, it is an equally big problem in plantation crops in south-west India. The common control measures that are prevalent in tea gardens and plantations are either cultural or chemical means. These methods are expensive and impracticable. Moreover, chemical herbicides can be very harmful to the non-target plants, people and the environment.

Between 1996 and 1999, surveys were conducted throughout the tropical and sub-tropical American native range of *M. micrantha* for pathogens having potential for CBC in India. Three microcyclic rust species, *P. spegazzinii*, *Dietelia portoricensis* (Whetzel and Olive) Buriticá and J.F. Hennen and *Dietelia mesoamericana* H.C. Evans and C.A. Ellison, were found to occur in association with the plant (Evans and Ellison, 2005). However, *P. spegazzinii* was selected for use as a CBC agent in India after an extensive host-range screening and studies on the environmental requirements for the fungus (Ellison, 2001). This rust is a microcyclic, autoecious species that infects all aerial parts of the plant causing necrosis and cankering, leading to plant death. These studies were carried out in the CABI Europe-UK quarantine in Ascot with funding from DFID, under a collaborative project between CABI and research institutions in India between 1996 and 2000.

*P. spegazzinii* was imported into the National Containment-cum-Quarantine Facility (CQF) for Transgenic Planting Material of the National Bureau of Plant Genetic Resources (NBPGR) in New Delhi during 2003 and 2004. After establishing the fungus at NBPGR, an additional host-specificity screening was undertaken during 2004 and 2005. This involved 74 plant species, including 18 species that were earlier tested in the UK, and reconfirmed the results from the UK: the rust is totally specific to *M. micrantha*. At NBPGR, the rust was found to be pathogenic to populations of mikania weed from several locations within Kerala and Assam, which indicated that *P. spegazzinii* has considerable potential as a CBC agent for mikania weed in India.

A Supplementary Dossier on the additional host-specificity tests provided the basis for obta ining the permit for release of *P. spegazzinii* from the Plant Protection Advisor to the Government of India, Ministry of Agriculture, in June 2005 (Kumar *et al.*, 2005). 'Limited' field releases of the rust have been made since 2005 in both Assam and Kerala (Ellison *et al.*, 2006; Sankaran *et al.*, in this volume). India has thus become the eighth country in the world to have released a plant pathogen for the CBC of a weed. This is also the first time that a fungal pathogen is being used as a CBC for mikania weed. An estimate made in 2004 indicates that more than 26 species of fungi originating from 15 different countries have been used as CBC agents against more than 26 weed species in seven countries (Barton, 2004).

The mikania weed CBC project in India has become a 'flag-ship' project. Other Asian countries, including China, are following the Indian example for future management of *M. micrantha* using *P. spegazzinii*.

The contrasts between the parthenium and mikania weeds fungal-CBC projects are presented in Table 3.

# Future strategies

## Parthenium weed

India is continuing work on both 'off-the-shelf' pathogens and arthropod natural enemies to increase the suppression of parthenium weed, already achieved by *Zygogramma bicolorata* Pallister. The seed-feeding weevil, *Smicronyx lutulentus* Dietz, is planned to be imported for the second time once the new quarantine facility being constructed at PDBC is functional. In addition, project funding will be sought to undertake strain selection studies of both rusts. For *P. abrupta* var. *partheniicola*, the aim is to identify a virulent strain that will be efficacious under Indian conditions and for *P. melampodii*, other strains need to be tested to see if there exists a strain that does not infect calendula.

There is also the option to investigate the potential of new agents, for example the white smut fungus, *Entyloma compositarum* de Bary. This fungus, capable of provoking severe leaf necrosis through the coalescing of grey, senescing lesions, was found in upland, humid, subtropical areas in Mexico and in semi-arid rangelands in Argentina by Evans (1997). Though this pathogen has not been evaluated as a CBC agent, it seems to have considerable potential (Kumar and Evans, 2005), especially in the light of the spectacular success of the closely related species, *Entyloma ageratinae* Barreto and Evans, against the highly invasive upland and cloud forest ecosystems weed mist flower [*Ageratina riparia* (Regal) R. King and H. Robinson] in Hawaii (Barreto and Evans, 1988), and more recently in both

**Table 3.** Contrasts between the parthenium and mikania weeds fungal-CBC projects.

| Parthenium weed project | Mikania weed project |
|---|---|
| Off-the-shelf agents available (previously released in Australia) | New agent identified and screened |
| Arthropod CBC agent already released in India | No previous CBC attempt in India |
| *Puccinia abrupta* var. *partheniicola* already present in India | No coevolved agents present in India |
| Non-target risk with *P. melampodii* | No non-target risk identified |
| Importation of rusts put on hold | Project led to release of rust in India |

South Africa and New Zealand (Evans *et al.*, 2001; Barton, 2004).

Overall, it is considered that an integrated approach is required for this weed, depending on the habitat it is invading and the level of control needed. For example, in peri-urban areas, a high level of control is necessary due to its toxicity to humans. Kumar and Evans (2005) stated: '…it is our considered opinion that management of parthenium weed in India will only be achieved through an integrated strategy based on biological control, specifically the classical approach with the introduction of host-specific or coevolved natural enemies from the plant's centre of origin/diversity in the Neotropics'.

### Mikania weed

The CBC project for mikania weed using *P. spegazzinii* is still in its early days since the release of the rust. However, there is a clear need to improve the rust release strategy to elicit an epidemic of the rust so it is in high enough concentrations to survive the dry season. CABI has seven strains of the rust under quarantine in the UK, and strains other than the one released in India may prove to be more aggressive under field conditions. In the future, it may be worth considering previously untried rust pathogens such as *D. portoricensis* and *D. mesoamericana* in new areas such as the Andaman and Nicobar Islands, or even in Assam and Kerala, if *P. spegazzinii* does not give substantial control of the weed. Finally, it should not be forgotten that substantial work has been undertaken on the arthropod natural enemies of mikania weed; the CBC potential of many were not fully investigated (Cock *et al.*, 2000).

## The way forward in India

### Infrastructure

A brand-new quarantine facility of international standards is being constructed on the PDBC campus in Bangalore, funded by ICAR. This two-storey containment facility will allow for an increase in entomological work on introduced natural enemies at PDBC. It will also have a pathogen-safe unit of level-4 containment (CL-4), to allow for the importation and screening of pathogens for the control of weeds and other pests. The upper floor or the 'Pathology' quarantine cell has two dedicated laboratories and a large greenhouse with three individual bays complete with cement platforms for plant propagation and handling. The entry to these is routed through a shower room sandwiched between two changing rooms to safeguard from entry of unwanted organisms and exit of organisms under quarantine. International standard air and water handling systems for quarantine facilities and equipment for waste disposal, viz. a double-ended autoclave and an incinerator, are integrated into the overall configuration. This facility is due to be finished and operational by early 2008.

Although the original aim was to undertake the mikania weed work in the PDBC facility, the CL-4 CQF on the NBPGR campus in New Delhi had to be used as an interim facility by PDBC for both the quarantining and host-specificity screening of *P. spegazzinii*. This facility also includes features such as outer and inner decontamination rooms provision for safe effluent treatment, a large incinerator and a dedicated generator as a stand-by for uninterrupted power supply are available.

With funds from the DFID mikania weed project, rust propagation units were constructed at the Assam Agricultural University (AAU, Jorhat) and the Kerala Forest Research Institute (KFRI, Peechi). These facilities have an area for plant propagation, an inoculation chamber and an area for rust infected plants to develop symptoms, prior to being placed in the field.

### Expertise

One of the major outcomes of the DFID-funded collaborative projects on *P. hysterophorus* and *M. micrantha* has been the development of local expertise in handing and quarantining exotic weed pathogens for biological control. The involvement of a host of institutes across the country in these projects has resulted in invaluable know-how and do-how expertise in India.

### Funding

Both the CBC projects on parthenium and mikania weeds were funded by DFID. Similarly, Indian government agencies, including ICAR and the Department of Biotechnology (DBT), have supported several research projects on biological control of weeds with pathogens, principally the mycoherbicide approach. Other international aid agencies operating in the Indian region may provide funding in future, e.g. the Australian Centre for International Agricultural Research (ACIAR). However, India is likely to have to look inward to national and regional funding in the future, as its economy continues to grow and the country becomes less dependent on external donor support.

### Process

Although India has a long history of importing CBC agents for the control of invasive alien weeds, until the mikania weed project, all the natural enemies had been arthropods or fish. The mikania weed project is considered a flagship project in India through which policy and procedures for the import and release of fungal CBC agents have been developed. This should enable easy passage of future agents through the regulatory system and into the field (Ellison *et al.*, 2005).

### Selection of future target weeds

Environmental weeds, both terrestrial and aquatic, should be the main targets of control through the classical

strategy (Kumar, 2005), although it is important to note that most environmental weeds also impact on agro-ecosystems and/or agroforestry.

The use of fungal pathogens in weed CBC is a relatively young technology compared to employing arthropods. Thus, it is not surprising that most weed targets for CBC, using fungal pathogens, are those where arthropods have not been effective, and this holds true for the major invasive alien plants in India.

## *Ageratina adenophora*

Crofton weed is not under control in India a gall fly has been released, but a suite of native parasitoids have prevented it from being effective. Other arthropods and pathogens have been identified, in the native range of the plant (Mexico), with good biocontrol potential, e.g., a lepidopterous and a curculionid stem borer and a rust fungus *Baeodromus eupatorii* (Arthur) Arthur. These agents have yet to be even established in the laboratory and thus are unlikely to be considered in the near future by India.

## *Chromolaena odorata*

A suite of fungal natural enemies have been documented from *C. odorata* in its native range (Evans, 1987a; Barreto and Evans, 1994), some of which have been partially assessed in the glasshouse (Elango *et al.*, 1993). However, the recent release of the stem gall fly, *Cecidochares connexa* (Macquart), against this weed in India (Bhumannavar *et al.*, 2007) means that no further agents will be considered in the short term.

## *Cyperus rotundus*

The grassy weed, purple nutsedge or nutgrass, *C. rotundus* L., which is broadly considered to be the world's worst weed (Holm *et al.*, 1977), hinders vegetable cultivation and is a huge problem in crops such as maize and sugarcane across India. It has an Old World centre of origin, possibly India, but the natural enemies do not seem to exert sufficient pressure to keep it under check in India, suggesting that its true centre of origin may be elsewhere.

Currently, a specific sub-project within the ICAR-funded network programme on 'Application of Micro-organisms in Agriculture and Allied Sectors' is being undertaken to develop a mycoherbicide-based strategy for its control. *Puccinia canaliculata* (Schwein) Lagerh (reported as *Puccinia romagnoliana* Maire and Sacc.), which is widely prevalent across India during winter, has been evaluated for augmentative use (Bedi and Sokhi, 1994). *Puccinia cyperi* Arthur and *Puccinia cyperi-tegetiformis* (Henn.) F. Kern, recorded in the Neotropics, and the *Uredo* spp., reported from the Old World (Barreto and Evans, 1995), are the pathogens that need to be explored for within India or considered for importation and evaluation.

## *Eichhornia crassipes*

Water hyacinth [*Eichhornia crassipes* (Martius) Solms-Laubach] was successfully controlled in many areas in India in the 1980s, but there have been some resurgence problems due in part to eutrophication of water bodies. There is a wealth of unexploited pathogens in its native range (Upper Amazon) (Evans, 1987a). Charudattan (1996) advocated research on the lifecycle, host-range and biocontrol efficacy of the rust *Uredo eichhorniae* Gonz. Frag. and Cif., which is found only in South America, as a high-priority area. This will of course necessitate significant investment, since a full (5 years +) project will be required.

## Invasive grasses

There are a number of grassy weeds of growing importance in India, for example littleseed canarygrass (*Phalaris minor* Retz.), originating from the Mediterranean, is a serious weed in wheat, and is developing resistance to certain herbicides. In addition, two species of *Echinochloa*, barnyard grass [*Echinochloa crus-galli* (L.) Beauv.] and jungle rice [*Echinochloa colona* (L.) Link.], are problematic in rice. Grasses are notoriously difficult targets for CBC because of their close relationship to staple crop species. However, some biotrophic pathogens are known to be species specific, within the Poaceae, and could be investigated for these two genera. For example, smuts and/or rusts have been recorded to infect species from both genera.

## *Lantana camara*

Lantana weed has had a wide range of natural enemies released to control it throughout its invasive range. Some have been more successful than others at suppressing the weed (Broughton, 2000; Day *et al.*, 2003). India is yet to invest significantly in studying the most successful agents and considering them for introduction. This included a leaf rust, *Prospodium tuberculatum* (Speg.) Arthur (ex Brazil) that was released in Australia in 2001 (Ellison *et al.*, 2006; Thomas *et al.*, 2006) and *Puccinia lantanae* Farl. (ex Peru) that attacks leaves, petioles and stems, which has been partially screened and would seem to have potential for control of lantana weed in India (Renteria and Ellison, 2004).

## *Mimosa diplotricha*

The giant sensitive plant, *Mimosa diplotricha* C. Wright, native to South America, has been selected by India for CBC using an off-the-shelf agent *Heteropsylla spinulosa* Muddiman, Hodkinson and Hollis. This psyllid is having a high impact on populations of this weed in Papua New Guinea and Australia and is soon to be imported into India (Kuniata and Korowi, 2001). Pathogens, such as the rust *Uredo mimosae-invisae* Viégas, may be worth future assessment.

### Phanerogamic parasitic weeds

Both hemi-parasitic and holo-parasitic weeds continue to cause enormous losses in several crops in India. *Striga* spp. interfere with cereals and legumes, whereas *Orobanche* spp. parasitise roots of solanaceous (particularly, tobacco) and asteraceous (e.g. sunflower) crops. *Cuscuta* spp. are equally problematic to ornamentals and trees. The dipteran, *Phytomyza orobanchia* Kaltenbach, imported into India from the former Yugoslavia, could not be field-released against *Orobanche* spp. because of problems in rearing of the fly. Both soil-borne and air-borne fungal pathogens described on *Striga* and *Orobanche* species are broad-range pathogens, excepting a few varieties or *formae speciales* of *Fusarium* species (Kroschel and Müller-Stöver, 2003). Highly host-specific pathogens still need to be collected in the centres of origin of these phanerogamic parasitic weeds.

## Conclusions

India has a fast-developing CBC of weeds programme that has now branched out and embraced pathogens as natural enemies. This strategy, therefore, finds an important place in the Perspective Plan ('Vision 2025') document of PDBC. The expertise, infrastructure and now the precedent set by importing and releasing the *M. micrantha* rust means that future projects are set to roll, with the process fully in place. There is a wealth of off-the-shelf arthropod and pathogen agents that could be fast-tracked for release over the next decade, targeting the most noxious weed species in India. In addition, many more pathogen agents have been identified that could be considered in the longer-term that require full assessment. However, significant financial support must be invested into this proven technology if the true potential is to be realized.

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# Explorations in Central Asia and Mediterranean basin to select biological control agents for *Salsola tragus*

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*Summary*

Russian thistle, *Salsola tragus* L., (Chenopodiaceae), a troublesome weed complex in the drier regions of western North America, is native to Central Asia and widely distributed throughout the Palaearctic Region. Since 2003, several exploration and survey trips to discover new potential biological control agents for the weed were carried out in Italy, Russia, Turkey, Tunisia, Kazakhstan, Greece, Egypt and Morocco. Twenty-five arthropod species (one gall midge, one heteropteran, two flea beetles, three moths and 18 weevils) and two fungi were preliminarily selected during the surveys. Among them, four arthropod species (two weevils and two moths) were selected for further biology and host-specificity studies. At the moment, the most promising among these is the stem-boring weevil *Anthypurinus biimpressus* Brisout. Preliminary host range no-choice tests and life cycle observations showed that this species is restricted for feeding, oviposition and development to Russian thistle.

***Keywords***: *Salsola*, Russian thistle, foreign exploration, tumbleweed, natural enemies.

## Introduction

Russian thistle or tumbleweed, *Salsola tragus* L., (Chenopodiaceae) is an invasive alien weed originating from Central Asia and is the target of classical biological control in the United States (Goeden and Pemberton, 1995; Pitcairn, 2004). This plant has commonly also been called *Salsola australis* R. Br., *Salsola iberica (Sennen and Pav)* Botsch ex Czereparov, *Salsola kali* L. and *Salsola pestifer* A. Nelson, and many synonyms occur in the literature (Mosyakin, 1996; Rilke, 1999). The taxonomy of Russian thistle is complicated because of high morphological variability of species in the genus and the occurrence of hybrids and polyploids; however, biochemical and molecular analyses are beginning to clarify the taxonomy. Similar species that are also invasive in North America include *Salsola paulsenii* Litv., *Salsola collina* Pallas, *Salsola* 'type B' and various hybrids (Ryan and Ayres, 2000; Akers *et al.*, 2003; Gaskin *et al.*, unpublished data). *S. kali* L also occurs in North America but is limited to seashores, primarily on the Atlantic coast (Mosyakin, 1996). In Eurasia, this species occurs primarily on seaside beaches of the Mediterranean and Atlantic coasts (Rilke, 1999), whereas *S. tragus* occurs primarily inland. All of these species are taxonomically closely related and have been placed in the *Salsola* section *kali*, sub-section *kali*, which is historically distributed across Eurasia (Rilke, 1999).

During a survey of genetic variability of *S. tragus* in California, a new variant, called 'type B' was discovered (Ryan and Ayres, 2000). Subsequent searches for the geographic origin of this population have revealed the same species only in South Africa and Australia. Until now, we have not been able to locate any specimens of Eurasian origin. Morphological and biochemical analyses support the restoration of this species to *S. australis* R. Brown (F. Hrusa, California Department of Food and Agriculture, personal communication). The origin of 'type B' is important to biological control because it differs significantly from *S. tragus* with re-

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spect to susceptibility to two prospective agents: a gall-inducing midge, *Desertovellum stackelbergi* Mamaev (Diptera: Cecidomyiidae; Sobhian *et al.*, 2003a,b) and a fungus, *Colletotrichum gloeosporoides* (Penz.) Penz. and Sacc. in Penz (Bruckart *et al.*, 2004). However, apart from 'type B', the other species all appear to originate from Eurasia and can be attacked by some of the same prospective biological control agents (Smith, 2005).

Foreign explorations to look for prospective biological control agents were previously conducted in Afghanistan, Pakistan, Egypt, Turkey, Uzbekistan, China and on the Mediterranean coast of France (Baloch and Mushtaque, 1973; Goeden, 1973; Hafez *et al.*, 1978; Sobhian *et al.*, 1999; Hasan *et al.*, 2001). As a consequence, two species of Coleophorid moths, *Coleophora klimeschiella* Toll, 1952, and *Coleophora parthenica* Meyr., 1891, were introduced in the 1970s to California and nearby states (Hawkes and Mayfield, 1976, 1978). These became widespread, but predators and parasites prevent them from becoming abundant enough to control the weed (Goeden and Pemberton, 1995). Other species that have been evaluated but were rejected for lack of specificity include *Lixus incanescens* Boheman 1836 [= *L. salsobe* Becker, 1867] (Coleoptera: Curculionidae), *Piesma salsolae* (Becker, 1867) (Hemiptera: Piesmatidae) and the fungus *Colletotrichum gloeosporioides* (Penzig) Penzig et Saccardo (Sobhian *et al.*, 2003a,b; Bruckart *et al.*, 2004). The blister mite *Aceria salsolae* DeLillo and Sobhian, 1996 (Acari: Eriophyidae) has been petitioned for permit to release (Smith, 2005), and *Gymnancyla canella* [Denis and Schiffermüller], 1775 (Lepidoptera: Pyralidae) is undergoing the final stage of evaluation before petitioning for release (Table 1). No other prospective agents worth evaluating were known. In the latest years, explorations were mainly targeted to Central Asia, the closest region to the probable centre of origin of *S. tragus*, and North Africa, which has the closest climatic match to the San Joaquin Valley of California, the locality where *S. tragus* is most problematic.

# Methods

## Field surveys

In 2004, the Biotechnology and Biological Control Agency (BBCA) began involvement in a program of explorations for Russian thistle natural enemies in the Mediterranean Basin and in Western and Central Asia. During the past 3 years, several surveys were carried out in Italy, North African countries, Turkey and Kazakhstan during late spring until late summer.

## Italy

Bibliography and herbarium surveys reported mainly *S. kali* in Italy: this Russian thistle species is found in this country only on undisturbed sandy sea shores. The weed is very common, mainly along the Central, Southern and island sandy beaches.

## North Africa

Similar habitat and weed species have been recorded in the three North African countries that we have inspected: Tunisia, Morocco and Egypt. All of them have

**Table 1.** Status of prospective biological control agents of Russian thistle.

| Taxonomic name | Common name | Current information |
|---|---|---|
| **Evaluated species** | | |
| *Aceria salsolae* (Acari: Eriophyidae) | Blister mite | The mite attacks developing tips. Petition approved by TAG, permit submitted to Animal and Plant Health Inspection Service (APHIS) |
| *Gymnancyla canella* (Lepidoptera: Pyralidae) | Seed and stem moth | Caterpillar feeds on seeds and young branch tips. Host specificity testing almost completed |
| *Lixus incanescens* [=*salsolae*] (Coleoptera: Curculionidae) | Stem weevil | Adults feed on many plants in choice test at Montpellier, France (Sobhian *et al.*, 2003a,b). Rejected |
| *Piesma salsolae* (Hemiptera: Piesmatidae) | Plant bug | Develops on beets in no-choice lab test at Montpellier, France (R. Sobhian, personal communication). Rejected |
| *Colletotrichum gloeosporioides* (Phyllachorales: Phyllacoraceae) | Fungus | More damaging to Russian thistle type A than to type B (Bruckart *et al.*, 2004). Being evaluated by W. Bruckart, US Department of Agriculture-Agricultural Research Service (USDA-ARS), Maryland |
| *Uromyces salsolae* (Uredinales: Pucciniaceae) | Rust fungus | Damages Russian thistle type A (Hasan *et al.*, 2001). Being evaluated by W. Bruckart, USDA-ARS, Maryland |
| **Newly tested species** | | |
| *Anthypurinus biimpressus* (Coleoptera: Curculionidae) | Jumping weevil | Found in Tunisia in 2004. Larvae and adults feed on leaves. Biology is unknown |
| *Baris przewalskyi* (Coleoptera: Curculionidae) | Weevil | Abundant on *Salsola* sp. in Kazakhstan in 2004. Biology is unknown |
| *Philernus sp.* (Coleoptera: Curculionidae) | Weevil | Found in Kazakhstan in 2004. Probably monophagous |

a peculiar combination of sandy beaches and sandy deserts. Among them, only in Tunisia was the target weed recorded in oases and in sandy areas at the beginning of the desert, while Russian thistle occurs in the other two countries only on the sandy dunes along the sea shore.

## Turkey

*S. kali* and *S. tragus* are both present in Turkey, but they occur in different areas and habitats. The first is always associated with sandy beaches along the Mediterranean coast, while the latter occurs in more rocky habitats, especially in the Central and Eastern Anatolia. Furthermore, climatic conditions are very different, with a classic Mediterranean climate along the coast and a continental climate in the interior regions.

## Kazakhstan

Explorations were carried out mainly in the south-eastern part of the country, in the region north of Almaty. Russian thistle was relatively common in disturbed areas, mainly along roads, in dense populations. Scattered populations were also found in wild fields. In both cases, the weed was associated only with sandy soils.

During the field explorations, the field sites where Russian thistle was present were recorded, adult insects were sampled, plants were dissected and eggs and immature larvae transferred into artificial diet for rearing. In addition, plant parts (leaves, shoots, roots) with immature stages or signs of mite or pathogen attack were collected and taken back to the laboratory, where immature stages were reared to adult and sent, together with field-collected adults, to taxonomists for identification.

# Results and Discussion

## Potential biological control agents

**Tunisia:** *Anthypurinus biimpressus* Brisout 1869 (Coleoptera, Cu culionidae): This stem- and leaf-mining weevil is recorded from one area in Tunisia (near Gabes) and was identified by one of us (E. Colonnelli). It is a univoltine species, and larvae attack the plant in May and June during the early phenological stages. From field observations, the insect has gregarious behaviour: the adults were found in 2004 and 2006 at two different sites near Gabes in large numbers on single individual plants. Almost all the Russian thistle plants at the sites were heavily attacked.

*Broconius biscrensis, Capiomont, 1874* (Coleoptera, Curculionidae): A weevil has been reported on Russian thistle in Central Tunisia according to a record in E. Colonnelli collection. Additional surveys are planned to collect and rear this weevil.

**Kazakhstan:** *Baris przewalskyi Zaslavskii* 1956 (Coleoptera, Curculionidae): This root-mining weevil was collected at one site in Kazakhstan (near Ushtobe). It has been identified by B. Korotyaev. The species was found as large number of adults resting and feeding on young shoots and around the crown of young Russian thistle plants. Unfortunately, the species has been found at one site only, together with three other oligophagous *Baris* spp. (*Baris sulcata* Boheman, 1836, *Baris convexicollis* Boheman, 1836 and *Baris memnonia* Boheman 1844), all very similar in appearance. In addition, *Cosmobaris scolopocea* (Germar, 1826) and *Elasmobaris signetera* (Faust, 1821) were found at the same spot.

*D. stackelbergi* Mamaev (Diptera, Cecidomyiidae): This stem-galling midge was found for the first time in Uzbekistan by R. Sobhian (EBCL). During 2004, two populations were recorded at two sites in south-eastern Kazakhstan. This family is reported to be extremely specific. Preliminary tests carried out by Sobhian in Uzbekistan confirmed the narrow host range of the species. Additional tests carried out at the USDA, Albany, CA, showed that gall formation may depend on the presence of an unknown symbiotic fungus (Biscet and Borkent, 1988).

## Other biological candidate agents

**Kazakhstan:** A weevil, *Philernus* sp., was collected in south-eastern Kazakhstan during 2004. Its biology is unknown, but preliminary bibliography surveys do not report any weevil of this genus associated with any crop plant.

During 2006, two populations of a root-boring moth were found in south-eastern Kazakhstan. The larvae were found in silken tunnels on the side of the roots, and feeding was observed on the external part of the root, near the place where the tunnel was affixed with the root system.

**Italy:** Several moths are reported to be closely related to Russian thistle (*S. kali*) in the Central Mediterranean Basin (A. Zilli, Museo Civico di Zoologia, Rome, Italy, personal communication). In particular, *Discestra sodae* Rambur, 1829 and *Discestra stigmosa* Christoph, 1887 (Noctuidae) are considered having a narrow host range within the genus *Salsola* and occur in Sardinia, Central Italian coasts, and Sicily. Similar distribution was also reported for several *Cardepia* spp., such as *Cardepia sociabilis* Graslin, 1860, *Cardepia deserticola* Rotschild, 1920, and *Cardepia hartigi* Parenzon, 1988. Moreover, a new species of *Gymnancila* sp. prope *canella* was found near Cefalù, northern Sicily. A population of *G. canella* from southern France is currently being evaluated for specificity (Sobhian, 2000; Smith *et al.*, 2007). Finally, noctuid moths of the genera *Lacanobia* Billberg, 1820 and *Pseudohadena* Alpheralay, 1889, even if not very common, are reported as oligophagous for the genus *Salsola*.

## Preliminary screening of new potential biological control agents

**Tunisia:** *Lixus sp.*: This weevil from eastern Tunisia is probably *L. incanescens*, already tested by R. Sobhian (EBCL) and shown to be *oligophagous*. We carried out a quick screening on newly collected live specimen in no-choice conditions. Feeding and in some cases oviposition have been recorded on all Chenopodiaceae species tested (common beet, sugar beet, Russian thistle). The insect was rejected.

*A. biimpressus* Brisout: This weevil from eastern Tunisia was found for the first time on *Salsola kali* near Gabès during late May 2004 (spring) and identified as a probable monophagous species. Larvae and/or adults feed on young leaves caused severe damage. Oviposition and larval development were obtained in the laboratory from adults collected in the field in spring, although larvae could not complete pupation. Preliminary laboratory observations showed that larvae appear to be external feeders; this is uncommon for weevils. The insect is likely univoltine because in 2006 at the Gabès site, no larvae or adults were seen in late June on plants that had been attacked in May. The generation collected in September (autumn) behaved differently from the one collected in spring. The autumn collected insects fed on host plant and mated but did not oviposit. Preliminary host range tests, carried out during 2006, tested four crop plants within the family Chenopodiaceae and indicate that the host range does not include species in the subfamily Chenopodioideae (e.g. *Chenopodium album* L., beets and spinach).

**Eastern Kazakhstan:** A wide range of arthropod fauna has been found associated with the target weed in Kazakhstan. The following species are likely monophagous: the weevil *Philernus* sp., the gall midge *D. stackelbergii* Mamaev and the weevil *B. przewalskyi* Zaslavskii. The following weevil species are likely oligophagous: *Lixus rubicundus* Zoubkoff 1833, *Lixus polylineatus* Petri 1900, *Lixus scabricollis* Boheman 1843, *C. scolopacea*, *B. sulcata*, *E. signifera*, *B. convexicollis*, *B. memnonia Temnorhinus elongatus* (Gebler 1845).

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# Eriophyoid mites on *Centaurea solstitialis* in the Mediterranean area

**R. Monfreda,[1] E. de Lillo[1] and M. Cristofaro[2]**

*Summary*

Yellow star thistle (YST), *Centaurea solstitialis* L. (Asteraceae), is a weedy annual plant, native to the Mediterranean area that infests rangelands, pastures and grasslands in the northern United States. In the search for biological control agents, an eriophyoid mite capable of significant impact on weed density and reproduction was studied. This research was focussed on: (a) geographical distribution of *Aceria solstitialis* de Lillo *et al.* (Acari: Eriophyidae) in the YST native area; (b) dynamic study of the eriophyoid population on YST in Apulia (Italy). In the first case, symptomatic plants were collected in Bulgaria, Greece, Italy and Turkey. In the second case, four sites were selected in Apulia, and periodical samplings were undertaken on 20 plants from February to August 2004 to 2006. Four different phenological categories of samples were collected separately from each plant: new rosette leaves, mature rosette leaves, stems and flower heads. This study ascertained the presence of *A. solstitialis* in the sampled localities of Mediterranean area, together with other mite species. In Apulia, ten species were detected on YST, and some of them could be related to neighbouring plants. *A. solstitialis* was detected only from May to August, and its density was highest at stem level, but it seems to be always low and insufficient to provide a significant reduction of weed seed production in the studied area.

***Keywords***: weeds, Eriophyoidea, population dynamics, geographical distribution.

## Introduction

Yellow star thistle (YST), *Centaurea solstitialis* L., is an annual species belonging to the Asteraceae family which has become a weed of extreme importance in several states of the USA, with the most serious infestations in Arizona, California, Idaho, Oregon and Washington (Maddox *et al.*, 1985). Native to the Mediterranean region of Europe and Southern Eurasia, this herbaceous plant probably arrived in the USA by way of contaminated alfalfa seeds (*Medicago sativa* L.) during the mid-1800s (Roché and Talbott, 1986; Roché and Roché, 1991) and actually represents a serious weed of pastures, rangelands, abandoned croplands, natural areas and roadsides (Lass *et al.*, 1996).

YST is an herbaceous winter annual plant propagating only by seeds that usually germinate in autumn or winter, depending on rainfall. In California, the transition from seedling to rosette occurs in late winter to late spring. The plant bolts during May and June to produce an erect and branched stem 20 to 75 cm in height, develops bud in mid-June to early July and flowers in mid-July to September. The spread and survival of this weed depend on seed production, and several thousand achenes may be produced per plant under optimal conditions (Maddox, 1981).

Several attempts have been made to find an efficient biological control agent (Piper, 2001). Recently, attention has been paid to mites (Acari: Eriophyoidea) as candidates for YST control (de Lillo *et al.*, 2003). Eriophyoid mites are considered extremely important for biological control of weeds because of their ability to damage the target plant sufficiently to reduce its impact, their high degree of host-specificity, their capacity for rapid population increase and their efficient dispersal (Briese and Cullen, 2001).

Two eriophyoids have been reported on *C. solstitialis*: *Aceria solcentaureae* de Lillo *et al.* and *A. solstitialis* de Lillo *et al.* (de Lillo *et al.*, 2003) with apparently similar effects on stem and flower head development. In particular, infested plants show reduced growth, stem apex and flower heads remain green and fresh during the hot-dry season, flower heads are less spiny and seedhead appear to be small in size.

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The aim of this research was to give a first contribution to the geographical distribution of *A. solstitialis* in the Mediterranean basin and to investigate dynamics of the eriophyoid population on YST in Apulia (Italy).

## Methods and materials

To study *A. solstitialis* geographical distribution, parts of symptomatic plants were collected in Greece (Alexandroupolis, Kilkis), Bulgaria (Plovdiv), Turkey (Goreme) and Italy (Apulia and Sicily) in 2001 and 2003 to 2006. Eriophyoids were extracted from fresh and dried materials, slide mounted and identified.

To investigate dynamics of eriophyoid populations, YST samples were randomly collected from four sites in Andria countryside (Italy). In 2004, 2005 and 2006, 20 plants were sampled from each site periodically at 30- and 15-day intervals from February to June and July to August, respectively. Four categories of samples, when available, were separately collected from each plant: new rosette leaf (with length  2 cm); mature rosette leaf (with length  10 cm); stem (without flower head); flower head. Mites were extracted by a 'washing and sieving method' (de Lillo, 2001; Monfreda *et al.*, 2007), identified, counted and recorded per part of plant. The collected samples were carefully inspected for detecting plant alterations related to eriophyoid activity.

## Results

*A. solstitialis* was recorded on YST in Bulgaria, Greece, Italy and Turkey, together with other species of eriophyoid mites (Table 1), some of which are related to YST. Few specimens were found for other species for which the identification was only at genus level. None of them fitted with the descriptions of species known to be associated with *Centaurea* spp.

In Apulia, ten species were detected on symptomatic yellow star thistle during 2004–2006. No eriophyoids were collected in February and March samplings, during the years of study. *A. solstitialis* was identified from May to August in sites 2 and 3 only, where other species were also present.

In 2004 samplings, *A. solstitialis* occurred from May to August. It appeared with very low population density in the first half of June on only new rosette leaves in sampling site 2 but both on new and mature leaves in site 3. It disappeared at the beginning of August on stems at site 2 and at the end of August on stem and flower heads at site 3. The highest population density was found at the beginning of July at level of stem at site 2 and 3, and in the early August on flower head before flower senescence (site 3).

In 2005 samplings, *A. solstitilis* was present on leaves at the end of May only in site 3, and on stems at the end of May in site 2 and longer in site 3 up to the

**Table 1.** List of the eriophyoids collected on *Centaurea solstitialis* in some countries of the Mediterranean area.

| Collection date | Collection locality | Species |
|---|---|---|
| June 2001 | Goreme–Cappadocia (Turkey) | *Aceria solstitialis* |
| July 2001 | Goreme–Cappadocia (Turkey) | *Aceria solcentaureae* |
| November 2001 | Goreme–Cappadocia (Turkey) | *Aceria solstitialis* |
| June 2003 | Kilkis (Greece) | *Aceria solstitialis* |
| June 2003 | Alexandroupolis (Greece) | *Aceria solstitialis* |
| June 2003 | Goreme–Cappadocia (Turkey) | *Aceria solstitialis* |
| | | *Aceria* sp. |
| June 2003 | Palermo–Sicily (Italy) | *Aceria* sp. |
| June 2003 | Piano Torre–Sicily (Italy) | *Aceria centaureae* |
| June 2003 | Mongerrati–Sicily (Italy) | *Aceria centaureae* |
| | | *Aceria solstitialis* |
| August 2004 | Plovdiv (Bulgaria) | *Aceria solstitialis* |
| April–August 2004 | Andria–Apulia (Italy) | *Aceria solstitialis* |
| | | *Aceria solcentaureae* |
| | | *Aceria centaureae* |
| | | *Aceria* sp. |
| | | *Aculodes* sp. |
| | | *Aculus* sp. |
| | | *Calepitrimerus* sp. |
| | | *Epitrimerus* sp. |
| | | *Eriophyes* sp. |
| | | *Trisetacus* sp. |
| April–August 2005 | Andria–Apulia (Italy) | *Aceria solstitialis* |
| | | *Aculodes* sp. |
| April–August 2006 | Andria–Apulia (Italy) | *Aceria solstitialis* |
| | | *Aceria* sp. |
| | | *Aculodes* sp. |

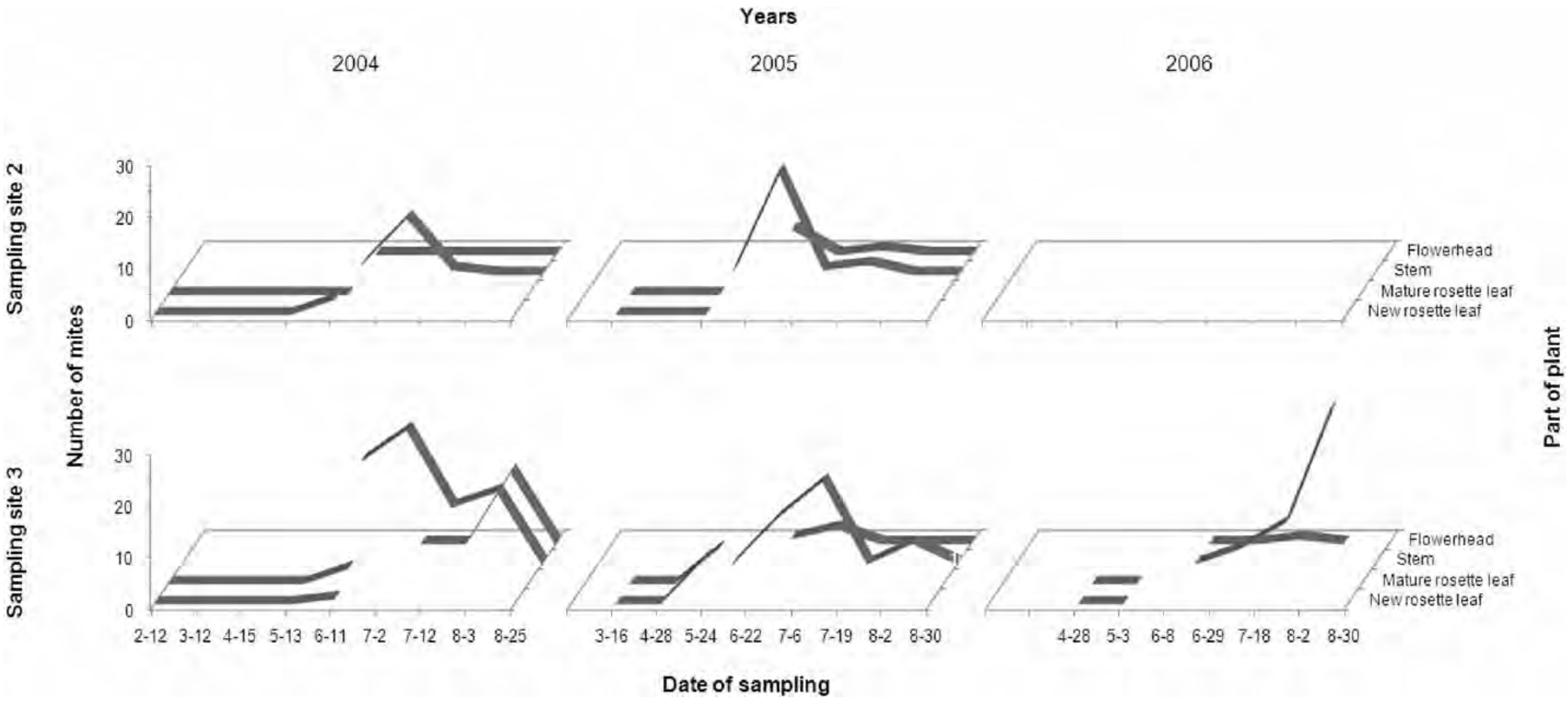


**Figure 1.** Population dynamics of *Aceria solstitialis* on *Centaurea solstitialis* during 2004, 2005 and 2006 in two sites in Apulia.

beginning of August. The highest density occurred in the second part of June in site 2 and in the first part of July in site 3, both at stem level.

Finally, in the 2006 samplings, this species was found only at site 3 from June to August, and its population density was higher at level of the stems at the beginning of August (Fig. 1).

## Discussion

A wide distribution of *A. solstitialis* in the Mediterranean basin was ascertained, together with other mite species. In Apulia, ten species were detected on YST, in the period of study (2004–2006). *Aculus* sp., *Calepitrimerus* sp., *Epitrimerus* sp., *Eriophyes* sp. and *Trisetacus* sp. could be related to neighbouring plants or they could have been dispersed by wind from other localities and other hosts because of their very low population density on *C. solstitialis* in the sampling sites. Specimens with similar morphometric features have not been found in the other countries of our study. *Aculodes* sp. and *Aceria* sp. could represent new species, but their host range needs to be verified.

Population density of *A. solstitialis* in 3 years of observations was highest at level of the stems in July and August samplings but always with very low values (at most 30 mites per sample). Moreover, the mite appeared in April and disappeared in August; no information on overwintering location was obtained. The hypothesis of mite dispersal by means of wind from other areas of the Mediterranean basin (i.e. Balcanian area), in coincidence of the highest density levels of the mite on the host, is highly reasonable (Sabelis and Bruin, 1996). The causes of their scarce density population on Apulian YST, while a large population was observed on YST from other areas, are still unknown. Possible explanations should be found in the genetic background of YST populations collected in different Mediterranean countries in case they could belong to different strains.

Even though *A. solstitialis* seems a promising agent for YST biological control, its Italian population seems to be insufficient to provide a significant reduction of seed production and requires further study.

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# *Diclidophlebia smithi* (Hemiptera: Psyllidae) a potential biological agent for *Miconia calvescens*

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*Summary*

*Diclidophlebia smithi* Burckhardt, Morais and Picanço (Hemiptera: Psyllidae) is a monophagous species which was selected as possible agent of biological control of miconia, *Miconia calvescens* DC. (Melastomataceae), a native plant of Central and South America that has become an aggressive invader of forest ecosystems in French Polynesia, Hawaii and Australia. The objective of this work was to study the biology and population dynamics of *D. smithi* in Viçosa and Dionísio (state of Minas Gerais, Brazil) from June 2001 to June 2002 and from February 2004 to February 2005 and evaluate injuries caused to the host plant and occurrence of natural enemies of this psyllid. Frequency distribution of the distance between the antennae indicated the existence of five nymphal instars. Colonies of the psyllid were observed throughout the year in Viçosa and Dionísio. Population peaks occurred from April to July (winter: a period of low temperatures, drought and short photoperiod in this region). Nymphs and adults were observed attacking buds, inflorescences and infrutescences of *M. calvescens* and causing damage by sucking the plant sap and injecting toxins. Desirable traits such as high population growth rate, easy mass rearing, occurrence throughout the year, host specificity, attack to reproductive organs and potential capacity to adapt to different climatic conditions including those similar to where *M. calvescens* invasions are occurring, all indicate that *D. smithi* is a promising biological control agent of *M. calvescens*.

***Keywords***: classical biological control, weed, Hawaii, population dynamics.

## Introduction

Miconia, *Miconia calvescens* DC. (Melastomataceae) is a plant native from Central and South America that became an aggressive forest ecosystem invader in French Polynesia and Hawaii (Meyer, 1996; Csurhes, 1997; Medeiros *et al*., 1997; PIER, 2002) and is also becoming a cause of concern in Australia (Csurhes and Edwards, 1998). It was introduced as an ornamental into Tahiti in 1937 and slowly invaded the native forests and now dominates 65% of the island (Meyer, 1996; Meyer and Florence, 1996; Meyer and Malet, 1997). Introduction into Hawaii occurred in the 1960s, and since 1992 it has been included in the list of noxious invasive weeds (Medeiros *et al*., 1997). It is now regarded as one of the hundred worst invasive weeds in the world (IUCN, 2007) and a serious threat to several large tropical forest ecosystems in the world, particularly in oceanic islands (Csurhes, 1997).

Classical biological control probably represents the sole sustainable alternative of control for invasive miconia populations. Studies were started in 1995 in Brazil aimed at finding pathogens to be used as classical biocontrol agents of miconia. Brazil is part of the centre of origin of *M. calvescens* and the country where the type material of the plant was first collected. Surveys were extended to Costa Rica, Dominican Republic and Ecuador. Results of the survey in Brazil were recently published (Seixas *et al*., 2007). Surveys for arthropod natural enemies of *M. calvescens* in Brazil started later in 2001 and concentrated in Viçosa, Dionísio and Guaraciaba, which are municipalities in the state of Minas Gerais. Over 70 species of arthropods were collected and identified, of which around 10 were recognized as having potential as biological control agents

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(Picanço *et al*., 2005). This included a newly described species of psyllid - *Diclidophlebia smithi* Burckhardt, Morais and Picanço (Hemiptera, Psylloidea) (Burckhardt *et al*., 2006).

*D. smithi* is a monophagous species that appears to be widely distributed in south-eastern Brazil. It was found in Dionísio, Guaraciaba and Viçosa in Minas Gerais State and Mangaratiba, Angra dos Reis (Ilha Grande) in Rio de Janeiro State. Psyllids are small sap-sucking insects that are typically highly specialized and either monophagous or oligophagous, often having the necessary attributes of a good biological control agent (Hollis, 2006). Nymphs and adults cause damage to their host plants either by sap sucking or toxin injection. This often leads to deformation of leaves and buds, necrosis, premature senescence of leaves and, in some species, to the formation of galls on infested plants. Some species of psyllids are also known to function as vectors of pathogenic phytoplasms and bacteria (Phyllis and Leann, 2006).

Before a biological control candidate can be further considered for use in a classical biological control program, a series of studies on its biology are regarded as necessary. These involve its precise identification, development of methods of raising it under controlled conditions, host-range evaluation, elucidation of life cycle, investigating its natural enemies and potential impact on host plant among others (Julien, 1997). This report presents the biology of *D. smithi* including life cycle, population dynamics, a description of damage caused to host plants and the occurrence of natural enemies.

## Materials and methods

### Life cycle

This study was performed at the Laboratório de Manejo Integrado de Pragas of the Departamento de Biologia Animal-Universidade Federal de Viçosa. Nymphs of *D. smithi* were brought from the field and transferred to young potted *M. calvescens* plants kept in a greenhouse.

To determine the number of instars during the life cycle of *D. smithi*, 20 new adult couples were placed on five *M. calvescens* plants (four couples per plant) and left there for egg-laying. Thirty nymphs obtained from the eggs were placed separately on young field-collected miconia leaves plus stem and maintained with the base of the stem immersed in water.

A daily evaluation was made of the following parameters: distance between the antennae, length of antennae and length and breadth of thorax at the level of the second pair of legs. Such measurements were made with the help of a dissecting microscope Leica MZ75 to which a digital camera was attached. Sizes were obtained through the analysis of photos taken with the camera with the software Leica Qwin. Additionally, body sizes of 40 adults (20 females and 20 males) and of 20 eggs were also measured.

Biometric data of the distance between the antennae served as the basis for the preparation of multimodal curves of frequency distribution aimed at determining the number of instars for *D. smithi.* Values of distance between the antennae were plotted on the abscissa axis, whereas the frequency of occurrence of each value was plotted on the ordinate axis. Each peak in the curve would then correspond to an instar in the insect's cycle (Parra and Haddad, 1989). Means and standard deviation for each biometric parameter, as well as for the duration of each stage, were calculated after the number of instars was determined.

These experiments were conducted at 20.4 ± 0.3°C, relative humidity of 77.3 ± 1.2% and daily light regime of 12.1 ± 0.04 h.

## Population fluctuation, injuries and natural enemies

Population density of *D. smithi* was evaluated in *M. calvescens* plants in Viçosa and Dionísio (state of Minas Gerais, Brazil). Viçosa is at 649 m of altitude, 20°45 14 S, 42°52 53 W. Mean temperature was 19.4ºC and precipitation 1221.4 mm/year. Dionísio is at 344 m of altitude and is located at 19°50 34 S, 42°46 36 W. Mean temperature was 23.2ºC and precipitation 1003 mm/year.

Evaluations were made during two different periods of time, first, from June 2001 to June 2002, and second, from February 2004 to February 2005. During the first period, evaluations were made at 3-week intervals at the two locations with a total of 15 evaluations at Viçosa and 16 at Dionísio. During the second period, evaluations were made at 15-day intervals in Viçosa and monthly at Dionísio, resulting in 27 evaluations in Viçosa and 13 in Dionísio.

Ten *M. calvescens* plants were selected randomly in each location, and the following plant organs (leaves, branches, buds, flowers and fruits) were evaluated per plant during each visit. Number of nymphs and adults of *D. smithi* and possible predators associated with the psyllid were recorded. The occurrence of *D. smithi* was also investigated on other plants surrounding each *M. calvescens* plant. Comparisons were made between attacked and healthy organs of miconia, particularly for the production of new growth, flowers, fruits, as well as the development of plant organs.

Climatic data for Viçosa were obtained from the Estação Climatológica Principal (INEMET/5º DISME/UFV), whereas those for Dionísio were obtained from the Estação Climatológica de Ponte Alta belonging to the private company CAF Santa Bárbara Ltda.

Occurrence of parasites on *D. smithi* was investigated on branches colonized by the psyllid that were collected at each visit to the sites and brought to the lab. These branches were left in plastic boxes with thin meshed screened openings. The base of those branches

was inserted in a layer of humid vermiculite over which a cardboard disk was placed separating the vermiculite from the upper chamber to avoid direct contact of the insects with the vermiculite. Branches were maintained for 3 weeks in the lab under room conditions. Appearance of parasitoids was evaluated at 2-day intervals.

Population fluctuation curves for nymphs and adults of *D. smithi* were prepared for each of the localities (Viçosa and Dionísio) for the two evaluation periods. Information about the phenology of *M. calvescens* over time was also recorded. Curves of recorded rainfall, average air temperature, relative humidity and photoperiod were also prepared.

# Results

## Life cycle

Eggs of *D. smithi* are pale yellow when laid and rapidly darken to black and shiny. They are elliptic with one pointed end and a peduncule attaching it to the plant. Its length is 0.24 ± 0.009 mm, and its diameter is 0.13 ± 0.003 mm (Table 1).

The frequency curve of the length between the antenae indicated that *D. smithi* has five instars (Figure 1). After egg-hatching, nymphs of the first instar are pale yellow and almost transparent and become black with time. Wing pads become visible during the third instar and third and fourth instars and are entirely yellow. In the fifth instar, the wing pads, as well as the last segment of the abdomen and the head become brown. Body and antennae length are given in Table 1.

Recently emerged adults are yellow with transparent wings that become brown a few minutes later. Females are bigger (1.99 ± 0.04 mm long) than males (1.71 ± 0.03 mm long; Table 1). A female can lay from 25 to 45 eggs during its lifetime in lab conditions (19 ± 2°C). Duration of each phase in the life cycle is presented in Table 1.

Nymphs of *D. smithi* secrete large quantities of whitish filamentous wax that can completely recover the colony, possibly protecting them against natural enemies, dehydration and rain.

**Table 1.** Biometric data and duration of stages in the life cycle of *Diclidophlebia smithi* (Hemiptera: Psyllidae).

| Life stage | Body | | Antenna | | Duration (days) |
|---|---|---|---|---|---|
| | Length (mm) | Width (mm) | Length (mm) | Distance between (mm) | |
| Egg | 0.24 ± 0.009 | 0.13 ± 0.003 | - | | 9.33 ± 0.09 |
| First instar | 0.26 ± 0.003 | 0.16 ± 0.003 | 0.12 ± 0.005 | 0.12 ± 0.003 | 3.09 ± 0.06 |
| Second instar | 0.32 ± 0.007 | 0.20 ± 0.004 | 0.14 ± 0.006 | 0.15 ± 0.002 | 3.76 ± 0.25 |
| Third instar | 0.42 ± 0.006 | 0.25 ± 0.003 | 0.19 ± 0.010 | 0.20 ± 0.002 | 5.13 ± 0.44 |
| Fourth instar | 0.63 ± 0.025 | 0.36 ± 0.010 | 0.35 ± 0.016 | 0.26 ± 0.003 | 4.50 ± 0.42 |
| Fifth instar | 1.00 ± 0.021 | 0.48 ± 0.009 | 0.50 ± 0.011 | 0.34 ± 0.005 | 7.09 ± 0.34 |
| Adult (male) | 1.71 ± 0.03 | 0.60 ± 0.008 | 0.60 ± 0.011 | - | 12.70 ± 0.45 |
| Adult (female) | 1.99 ± 0.09 | 0.62 ± 0.014 | 0.59 ± 0.014 | - | 12.00 ± 0.36 |

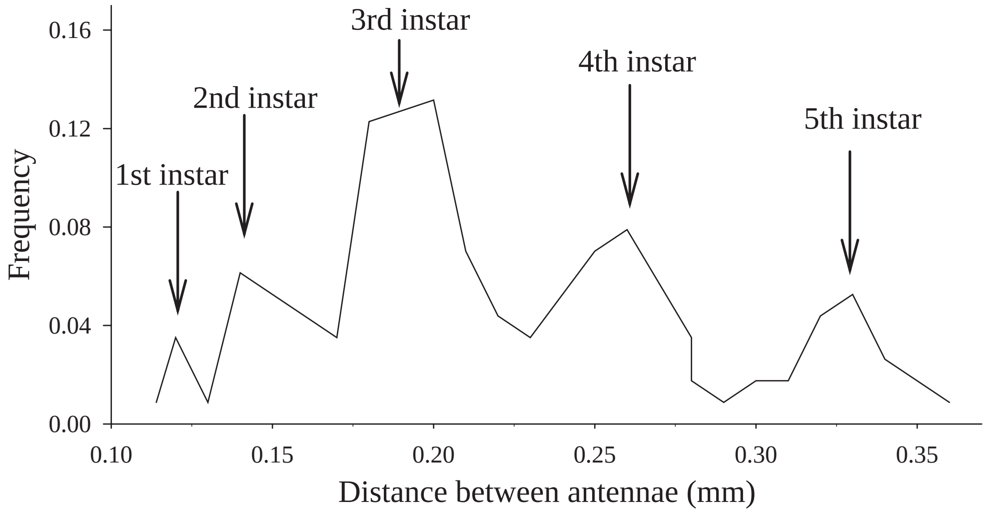


**Figure 1.** Frequency of distance between antennae in nymphs of *Diclidophlebia smithi* (Hemiptera: Psyllidae).

## Population fluctuation, injuries and natural enemies

The highest degree of damage caused by *D. smithi* to *M. calvescens* happened at high colony densities on buds, flowers and fruits. In such situations, the buds and young leaves became distorted and yellow, and their growth was hampered.

Nymph populations peaked from April to June of 2002 in Viçosa, coinciding with the period of the year when there is abundant new growth. In Dionísio, the population peak occurred in July of 2004 during a period of abundant fruiting. The highest values for population densities for adults occurred in the first year of evaluation, in July 2002, during a period of abundant fruiting and flowering. In the second year of evaluation, the peak occurred in April coinciding with the end of a fruiting period when new growth was produced. Those population peaks coincided with periods of cool weather, short photoperiod and drought (Figures 2and 3).

The only predator found attacking *D. smithi* nymphs was an unidentified Syrphidae larva. It was seldom

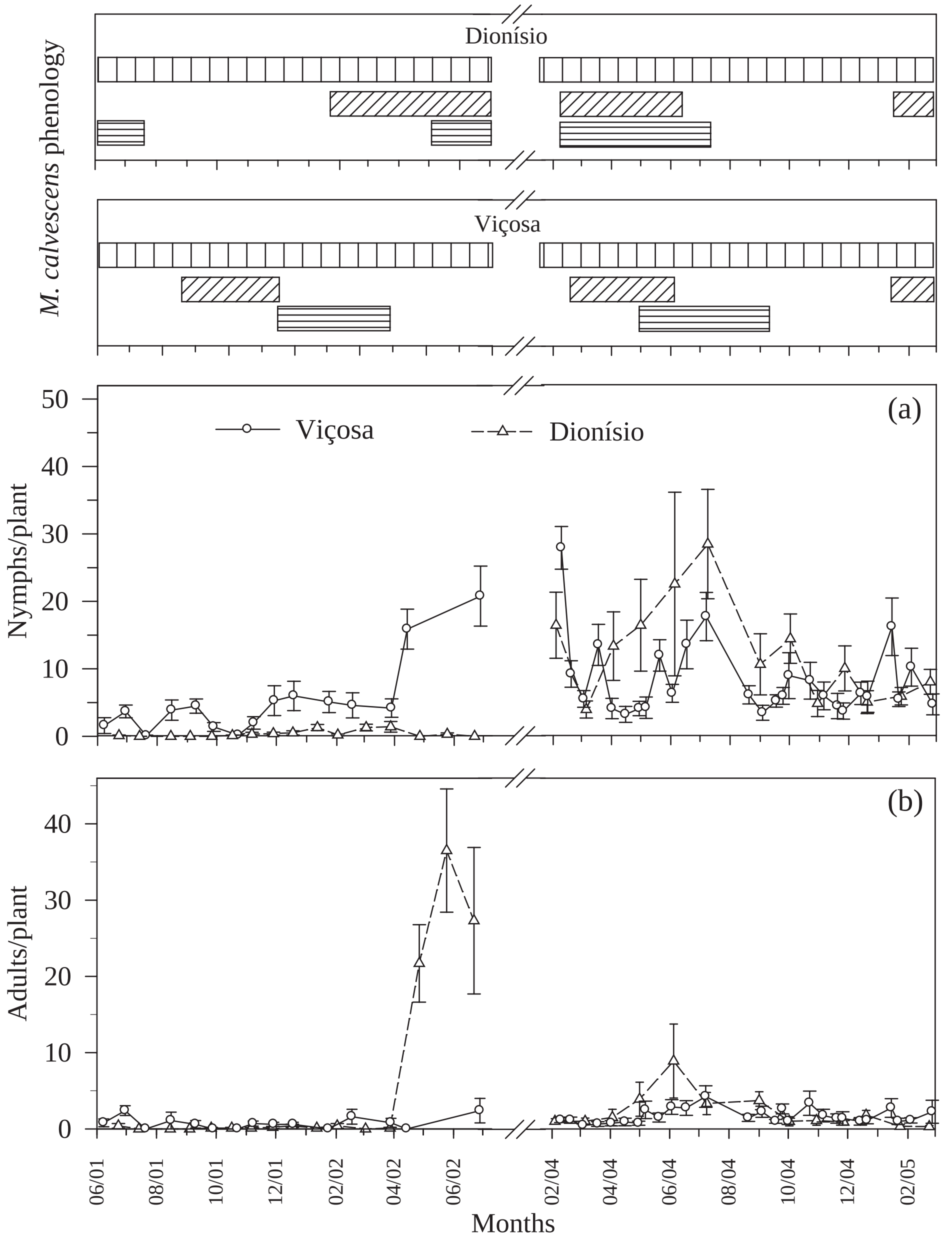


**Figure 2.** Phenology of *Miconia calvescens*: *vertical stripes* bud emission; *diagonal stripes* flowering; *horizontal stripes* fruiting, and population density of nymphs (a) and adults (b) of *Diclidophlebia smithi* (Hemiptera: Psyllidae) in Dionísio and Viçosa (state of Minas Gerais-Brazil), 2001–2002 and 2004–2005. *Vertical lines* on the population density graphs represent the standard error for the means.

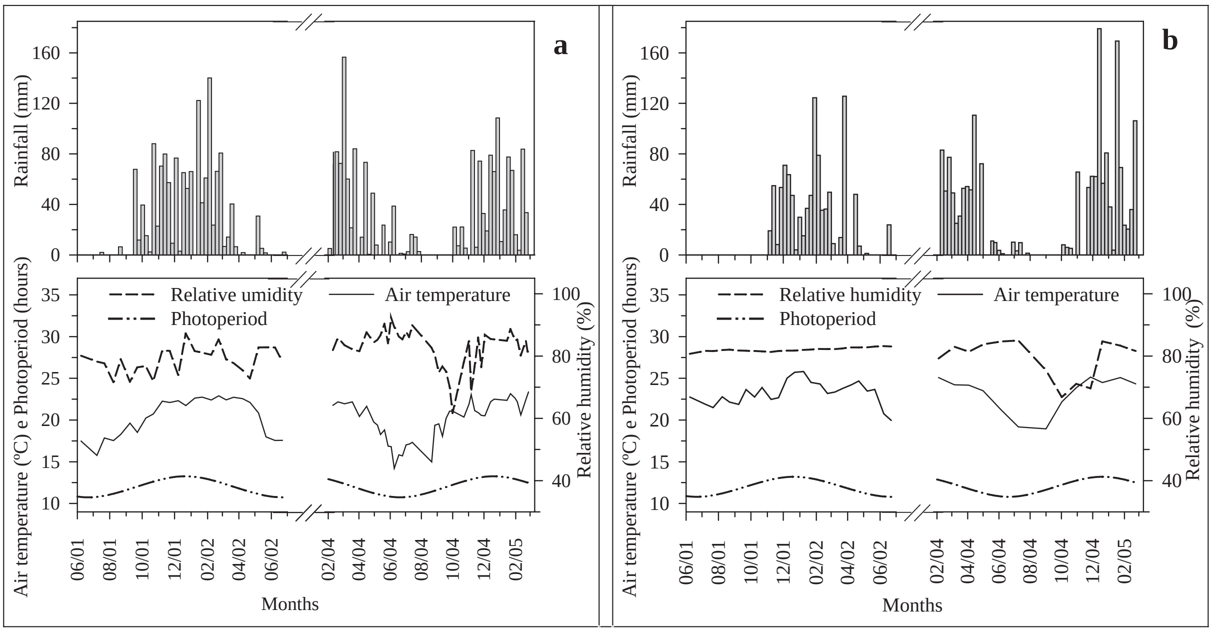


**Figure 3.** Climatic data: precipitation, relative humidity and photoperiod in Viçosa (a) and Dionísio (b), state of Minas Gerais, Brazil, 2001–2002 and 2004–2005.

found in the field, but it was observed to be a very voracious predator. No parasitoid was obtained during the investigation of colonies brought from the field.

## Discussion

Psyllid population peaked from April to June indicating that the density of this insect was related to a high abundance of food such as buds, flowers and fruits that formed during that period. Soft tissues are of critical importance for population growth of this sap-feeding insect. Similar connection of phenological states in plants and insect population density was also observed for other species in the Psyllidae such as *Euphalerus clitoriae* (Burckhardt and Guajará, 2000) *Diaphorina citri* Kuway, *Diclidophlebia lucens (*Burckhardt *et al*., 2005) and *Boreioglycaspis malaleucae* Moore (Burckhardt and Guajará, 2000; Tsai and Liu, 2000; Burckhardt *et al*., 2005; Center *et al*., 2006).

The highest population densities of *D. smithi* also coincided with periods of cooler air temperatures, shorter photoperiods and lower rainfall levels. Air temperature emerged as the climatic factor that had the highest influence on population density. High temperatures may harm the development of *D. smithi*, particularly when relative humidity is low. This was also observed for other Psyllidae such as *Euphalerus vittatus* Crawford on *Cassia fistula* L. (Fabaceae) and *E. clitoriae (*Burckhardt and Guajará, 2000) on *Clitoria fairchildiana* Howard. (Fabaceae) (Sahu and Mandal, 1999; Gondim Junior *et al*., 2005). Air temperature is one of the most important factors interfering with establishment, proliferation, dispersal and impact of organisms in new areas of distribution (Baker, 2002). High air temperatures cause a high mortality of nymphs and a reduction in egg-laying for female psyllids because of abnormal development of ovaries (Mehrnejad and Copland, 2005; Stratopoulou and Kapatos, 1995; Liu and Tsai, 2000). Adult *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) had maximum rates of egg-laying in lab conditions at 20°C and a reduction in ovary development when temperatures were above 30°C (Mehrnejad and Copland, 2005). High air temperatures can also reduce survival and longevity of psyllids as observed for *Trioza hirsuta* Crawford (Hemiptera: Psyllidae) which had a longer period of survival for adults at 25°C (Dhiman and Singh, 2003) and *D. citri*, for which the ideal temperature is around 20°C (Liu and Tsai, 2000).

Rainfall also influences population density of *D. smithi*. Rain can cause mortality through mechanical impact on individuals and can remove individuals from their host plant or, in the case of nymphs, remove the wax cover that protects them (Center *et al*., 2006), exposing them to adverse environmental conditions. Rain may harm psyllid mating and movement, leading to a reduction in population growth. Photoperiod may have an indirect effect on this insect's populations. Periods of adverse environmental conditions do not appear to represent a significant obstacle for this psyllid species since colonies of *D. smithi* recover readily after unfavourable periods, and colonies are seen in the field throughout the year.

Knowledge about effects of climate on a potential biocontrol agent is of critical importance in order to estimate its chance of success and to choose the correct

time of the year for its introduction into the field. In the case of *D. smithi* as a biocontrol for *M. calvescens*, the ideal time of the year would be a period of lower rainfall levels and cooler air temperatures and when there was plenty of new growth.

The attack of *D. smithi* to buds of *M. calvescens* is likely to effect plant development through starvation of such new organs of photoassimilates brought in the sap. The mass of wax filaments appear to have a deleterious effect on the formation of normal flowers and fruits. If seed production is affected, the impact might be important since *M. calvescens* relies entirely on seeds for its dispersal (Meyer, 1998). In the case of citrus attacked by *D. citri*, a reduction on seed setting has been demonstrated (Michaud, 2004). More detailed impact studies are presently being performed for *D. smithi* on *M. calvescens.*

Features of *D. smithi* such as it being relatively easily amenable to mass rearing under controlled conditions, its occurrence in the field throughout the year, the fact that it attacks the reproductive organs of the plant, its high host specificity (discussed elsewhere) and its ability to adapt to a range of climatic conditions indicates that it has good potential as a classical biological control agent to be used against *M. calvescens.*

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# A lace bug as biological control agent of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae): an unusual choice

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*Summary*

The lace bug *Tingis grisea* Germ. (Hemiptera: Tingidae) is a univoltine sap-feeder associated with the genus *Centaurea* L. and distributed throughout Central and Southern Europe and the Middle East. In 2002, one Turkish population of *T. grisea* was selected as a potential biological control agent for yellow starthistle, *Centaurea solstitialis* L., (Asteraceae: Cardueae), a weed of primary concern in the USA. Field observations showed that significant damage was caused to the host plant especially when many individuals were feeding on the same plant. Life-cycle and biology observations were made to assess the duration of the five nymphal instars of *T. grisea* under laboratory conditions, as well as female fecundity and longevity. Starvation and oviposition no-choice tests were carried out in order to determine the host specificity of the insect. Results showed a clear oligophagous behaviour closely restricted to the genus *Centaurea.* In addition, among the three *Centaurea* spp. on which full larval development was ascertained (*C. solstitialis*, *Centaurea sulphurea*, *Centaurea cyanus*), yellow starthistle was clearly most suitable regarding number of eggs laid and number of adults obtained.

***Keywords***: YST, host range, *Tingis grisea.*

## Introduction

Yellow starthistle, *Centaurea solstitialis* L., (Asteraceae: Cardueae) is an important invasive alien weed of rangeland in the western United States and is the target of a USDA classical biological control program (Turner *et al*., 1995; Sheley *et al*., 1999; Smith, 2004; Di Tomaso *et al*., 2006). Six insect agents that attack *C. solstitialis* flowerheads have already been introduced (Cristofaro *et al*., 2002; Pitcairn *et al*., 2004), but they do not appear to be reducing the weed population sufficiently (Pitcairn *et al*., 2000, 2006). Therefore, it is desirable to find new agents that attack other organs of the plant or earlier phenological stages. A rust, *Puccinia jaceae* var. *solstitialis* Otth., has been released (Woods, 2004; Fisher *et al*., 2006), and two beetles are being evaluated (Cristofaro *et al*., 2004; Smith, 2004; Smith, 2007); all of these attack immature plants. However, it would also be useful to have an agent that stresses the plant later in the growing season, during the critical period when it is flowering and producing seed (Smith, 2004). During foreign exploration for new biological control agents in eastern Turkey in 2002, we discovered a large population of the lace bug *Tingis grisea* Germ. 1835 (Hemiptera: Tingidae) feeding on mature *C. solstitialis* rosettes and on bolting plants.

In the literature, this lace bug has been reported from 11 species of *Centaurea*, including *C. solstitialis*, as well as from *Crupina vulgaris* L. (Stusak, 1959), a very closely related plant species (Susanna *et al*., 1995). Its geographical distribution is very wide: it occurs from central Spain across southern Europe to southern Russia; generally overlapping the distribution of *C. solstitialis* and some close relatives (Komarov, 1934; Klokov *et al*., 1963; Wagenitz, 1975; Dostál, 1976). Little is known about its biology (Stusak, 1959; Péricart, 1983). *T. grisea* is reported as univoltine and overwinters as adult. Oviposition begins in May, and

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eggs are inserted into the young tissue of stems and axils. Eggs are very small; the operculum measures 0.16 0.05 mm (Stusak, 1957). In Ukraine, nymphs appear at the beginning of June and adults at the beginning of July. Five nymphal instars have been described.

This paper reports results of studies on the life cycle, rearing and host-plant specificity of this insect to determine whether it warrants further evaluation as a candidate for biological control of yellow starthistle.

# Methods and materials

## Collection of insects

Insects emerging from winter diapause were collected in late March of 2004, 2005 and 2006 in the vicinity of Horasan (Erzurum Region, 1600 m ASL), Eastern Turkey. In the laboratory, lace bugs were kept in a 3-l glass beaker at low temperature (8°C) and 12:12 h L/D photoperiod. Insects were allowed to feed on freshly cut leaves of *C. solstitialis* (US biotype) held in water vials; crumpled tissue paper was also provided as shelter for insects to rest.

## Laboratory rearing

Insect rearing was carried out on natural substrate, using potted plants of *C. solstitialis* (US biotype) at 23°C to 26°C and 16:8 h L/D. Single pairs of *T. grisea* were confined to a portion of yellow starthistle stem anchored in a foam disk on the bottom of a 17 5 cm transparent plastic cylinder, capped with fine nylon mesh. A hole in the side, closed by a foam plug, was used for insect manipulation. After 7 days, insects were removed and transferred to another stem under the same conditions. Beginning 10 days after insect exposure, stems were cut off and daily examined under a stereo microscope to search for neonate nymphs. The same procedure was repeated several times for each pair of insects. Emerged nymphs were used for host-range, larval transfer experiments and life-cycle observations.

## Host specificity

The host specificity of the insect was assessed by means of no-choice tests on plant species related to yellow starthistle, including US native and US commercial crops.

## No-choice oviposition experiment

In 2004, stems of *C. solstitialis* (US biotype) and other test plants were exposed to a pair of insects in transparent cylinders at 23°C to 26°C and 16:8 h L/D. After 3 to 4 days, insects were removed and stems observed under the stereo microscope in order to count eggs. Because of the extremely small size of egg opercula and the complexity of stem features (tissue foldings and richness of trichomes), it was impossible to count eggs. Thus, we indirectly evaluated oviposition success by retrieving emerged nymphs from stems exposed to insects. In 2006, insects were tested on leaves instead of stems. In fact, preliminary trials carried out at the beginning of the season showed that *T. grisea* is able to lay eggs both on leaves and on stems. In this way, we were able to see eggs by observing leaves under the stereo microscope with backlighting. Leaves with eggs were stored in a plastic box on tissue paper until nymph emergence. Each pair was kept on yellow starthistle before and after being tested on any other plant species in order to give the insects the possibility to feed on the host plant and to be sure that females were actually ovipositing. Replicates on test plants were considered invalid if the continuity of a female's oviposition ability could not be demonstrated on *C. solstitialis* after a replicate with zero eggs on any non-host plant. Two to 11 specimens for each plant species were tested in 2004 and 2006; the plants tested are listed in Table 1.

## Larval transfer experiment

Nymphs of *T. grisea* (two to six per replicate) were transferred to intact leaves of potted *C. solstitialis* and other test-plant species, confined in transparent plastic cylinders at 23°C to 26°C and 16:8 h L/D. The first observation occurred after 7 days in order to assess nymphal development and mortality. Afterwards, observations were carried out every 3 to 4 days until all the nymphs either reached the adulthood or died. For each plant species, we tested an average of five specimens. Plants tested from 2004 to 2006 are listed in Table 2.

# Results and discussion

## Life cycle

Life-cycle observations, carried out during laboratory rearing and oviposition and larval transfer trials, show that under laboratory conditions (23°C to 26°C, 16:8 h L/D), first-instar nymphs emerged 10 to 12 days after oviposition. The duration of the first and second larval stages was approximately 3 to 4 days, while the development of each stage, from third to fifth instars, took 7 to 8 days. Total development was approximately 31 days (Figure 1).

## No-choice oviposition experiment

Results of no-choice oviposition tests, performed in 2004 and in 2006, clearly showed that *T. grisea* oviposits most on *C. solstitialis* and, with limited success, on closely-related species (Table 1), including *Centaurea stoebe*, *Centaurea cyanus*, *Centaurea diffusa*, *Centaurea sulphurea* and *Acroptilon repens*. In 2004, oviposition occurred on only three of the eight plant species tested. The number of larvae that emerged per replicate

**Table 1.** Summary of no-choice oviposition tests carried out in 2004 and 2006.

| | 2004 No-choice oviposition | | | | | 2006 No-choice oviposition | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of plants tested | Number of emerged larvae | Valid replicates | Non-valid replicates | Number of larvae/valid replicates | Number of plants tested | Number of eggs laid | Number of emerged larvae | % emerged larvae | Valid replicates | Non-valid replicates | Number of eggs/valid replicates | Number of larvae/valid replicates |
| Subtribe: Centaureinae | | | | | | | | | | | | | |
| *Acroptilon repens* | | | | | | 3 | 5 | 0 | 0 | 3 | 0 | 1.7 | 0 |
| *Carthamus tinctorius - Linoleic* | 5 | 0 | 3 | 3 | 0 | 6 | 0 | 0 | 0 | 2 | 5 | 0 | 0 |
| *Carthamus tinctorius - Oleic* | 5 | 0 | 3 | 3 | 0 | 5 | 0 | 0 | 0 | 2 | 3 | 0 | 0 |
| *Crupina vulgaris* | 3 | 0 | 2 | 1 | 0 | | | | | | | | |
| Genus: Centaurea | | | | | | | | | | | | | |
| *Centaurea cyanus* | 3 | 4 | 3 | 1 | 1.3 | 5 | 4 | 1 | 25 | 2 | 4 | 2 | 0.5 |
| *Centaurea diffusa* | | | | | | 3 | 6 | 1 | 17 | 3 | 0 | 2 | 0.3 |
| *Centaurea stoebe* | 4 | 5 | 2 | 2 | 2.5 | | | | | | | | |
| *Centaurea melitensis* | | | | | | 3 | 0 | 0 | 0 | 2 | 1 | 0 | 0 |
| *Centaurea solstitialis* | 16 | 22 | 25 | 19 | 0.9 | 19 | 266 | 106 | 40 | 59 | 19 | 4.5 | 1.8 |
| *Centaurea sulphurea* | | | | | | 2 | 3 | 0 | 0 | 2 | 0 | 1.5 | 0 |
| Subtribe: Carduinae | | | | | | | | | | | | | |
| *Carduus pycnocephalus* | | | | | | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| *Cirsium brevistylum* | | | | | | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| *Cirsium hydrophilum* | | | | | | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| *Cirsium loncholepis* | | | | | | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| *Cirsium occidentale* | | | | | | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| *Cynara scolymus* | 4 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| Tribe: Heliantheae | | | | | | | | | | | | | |
| *Helianthus annuus* | | | | | | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |

**Table 2.** Summary of the larval transfer results conducted during 2004 to 2006.

| | Stage of larvae transferred [a] | | | | | | | Total plants tested | Total larvae transferred |
|---|---|---|---|---|---|---|---|---|---|
| | L1 or L2 | | | | L3 or L4 | | | | |
| | Number of plants tested | Number of larvae transferred | % developed L3 | % developed adult | Number of plants tested | Number of larvae transferred | % developed adult | | |
| Subtribe: Centaureinae | | | | | | | | | |
| *Acroptilon repens* | | | | | | | | | |
| *Carthamus tinctorius - Linoleic* | 5 | 30 | 0 | 0 | 7 | 29 | 0 | 12 | 59 |
| *Carthamus tinctorius - Oleic* | 6 | 33 | 6 | 0 | 6 | 31 | 3 | 12 | 64 |
| *Crupina vulgaris* | 3 | 18 | 0 | 0 | 1 | 10 | 0 | 4 | 28 |
| Genus: Centaurea | | | | | | | | | |
| *Centaurea americana* | 2 | 20 | 0 | 0 | | | | 2 | 20 |
| *Centaurea cyanus* | 5 | 27 | 56 | 41 | 7 | 28 | 29 | 12 | 55 |
| *Centaurea diffusa* | 2 | 10 | 30 | 0 | | | | 2 | 10 |
| *Centaurea stoebe* | 2 | 10 | 30 | 0 | 2 | 10 | 0 | 4 | 20 |
| *Centaurea melitensis* | | | | | | | | | |
| *Centaurea rothrockii* | | | | | | | | | |
| *Centaurea solstitialis-CA* | 6 | 32 | 81 | 63 | 2 | 15 | 73 | 8 | 47 |
| *Centaurea sulphurea* | | | | | 3 | 20 | 25 | 3 | 20 |
| Subtribe: Carduinae | | | | | | | | | |
| *Carduus pycnocephalus* | | | | | | | | | |
| *Cirsium brevistylum* | 1 | 10 | 0 | 0 | | | | 1 | 10 |
| *Cirsium cymosum* | 2 | 10 | 10 | 0 | | | | 2 | 10 |
| *Cirsium hydrophilum* | 2 | 10 | 0 | 0 | 4 | 16 | 0 | 6 | 26 |
| *Cirsium loncholepis* | 2 | 10 | 0 | 0 | 4 | 16 | 0 | 6 | 26 |
| *Cirsium occidentale* | 1 | 10 | 20 | 0 | | | | 1 | 10 |
| *Cirsium vinaceum* | 2 | 10 | 0 | 0 | | | | 2 | 10 |
| *Cynara scolymus* | 4 | 26 | 0 | 0 | 1 | 10 | 10 | 5 | 36 |
| Tribe: Heliantheae | | | | | | | | | |
| *Helianthus annuus* | 1 | 10 | 0 | 0 | 4 | 17 | 0 | 5 | 27 |

[a] *L1* First-instar nymphs, *L2* second-instar nymphs, *L3* third-instar nymphs, *L4* fourth-instar nymphs.

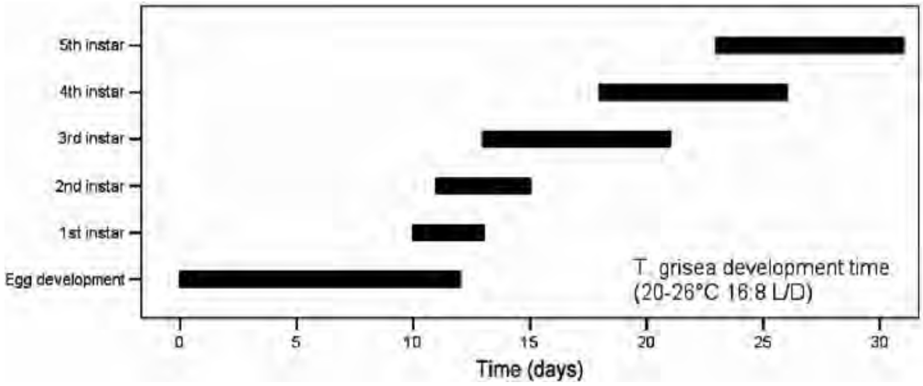


**Figure 1.** Diagram of estimated development time of immature stages of *Tingis grisea* under laboratory conditions (23 ± 3°C, 16:8 h L/D photoperiod).

was 0.9 on *C. solstitialis*, 1.3 on *C. cyanus* and 2.5 on *C. stoebe*. The total number of nymphs emerged was considerably higher on yellow starthistle, although it was very low compared to the number of replicates carried out (Table 1). Lack of knowledge about development times of the insect in this preliminary phase, in addition to a relatively low survival rate of the eggs on cut stems, was probably responsible for this low number of nymphs recorded.

In 2006, females laid nearly 94% of their eggs on yellow starthistle, and 40% of them produced nymphs. The relatively low emergence rate can be attributed to low egg survival on cut leaves due to rapid withering and occurrence of mould. Although we found eggs on *C. cyanus*, *C. diffusa*, *C. sulphurea* and *A. repens*, nymphs emerged only from eggs laid on *C. cyanus* and *C. diffusa*. In both *C. sulphurea* and *A. repens*, leaves with eggs became mouldy several days after oviposition, thus further experiments are needed to improve the measure of nymphal emergence on these plants.

### Larval transfer experiment

In larval development no-choice tests carried out from 2004 to 2006, nymphal survivorship was greatest on yellow starthistle (Table 2): 63% of first and second instar nymphs and 73% of third and fourth instar nymphs reached the adult stage on *C. solstitialis*, while a smaller percentage was able to complete the development on a small number of closely related species (*C. cyanus* and *C. sulphurea*). In general, development and survival was greater for old nymphs than for young ones, on both the target and nontarget plants. We observed development of one adult from 3rd instar on an oleic variety of *Carthamus tinctorius* and one on *Cynara scolymus*, but none of the younger (1st and 2nd instar nymphs) became adults. This insect is not a pest of either of these crops, which suggests that transfer of 1st and 2nd instars is more valid than transfer of 3rd and 4th instars.

## Conclusions

Preliminary host-specificity results, obtained from 2004 to 2006, showed clear oligophagy by *T. grisea*. Among the species on which oviposition occurred (*C. cyanus*, *C. stoebe*, *C. diffusa*, *C. sulphurea* and *A. repens*), the target weed *C. solstitialis* was clearly preferred in terms of number of eggs laid and number of nymphs emerged. In addition, only a few nymphs that were transferred to non-target plants completed development on species closely related to yellow starthistle (*C. cyanus* and *C. sulphurea*). 3rd instar nymphs could sometimes develop on critical nontarget plants, such as *C. tinctorius* and *C. scolymus*, but transfer of 3rd and 4th instar nymphs represents an extreme situation that is not likely to occur in the field because the nymphs are not highly mobile. Failure of young larvae to develop on *C. americana* is very promising because this is the closest native North American relative to the target weed.

Further feeding and oviposition trials under no-choice and choice conditions are required to better define the host range of this insect and understand if it represents a good candidate for the biological control of *C. solstitialis*. Moreover, laboratory tests and open-field trials are needed to evaluate its impact on the target species.

## Acknowledgements

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# Pathogens from Brazil for classical biocontrol of *Tradescantia fluminensis*

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*Summary*

*Tradescantia fluminensis* Vell., also known as wandering Jew, is an herbaceous monocot native to South America. It is an invasive plant in New Zealand and the south-eastern United States where it is considered highly invasive by the Florida Exotic Pest Plant Council. The pathobiota of *T. fluminensis* in Brazil is almost unknown and could include phytopathogenic microorganisms that could be used in classical biological control programs. A survey for specialized, coevolved phytopathogenic microorganisms of *T. fluminensis* was initiated in 2003. Five fungal species have been collected including three basidiomycetes—a rust fungus (*Uredo* sp.), *Kordyana tradescantiae* (Pat.) Racib. and *Ceratobasidium* sp.; a hyphomycete—*Cercospora apii* Fresen. and an ascomycete—*Mycosphaerella* sp. A bacterial disease was also observed and the bacterium identified as *Burkholderia andropogonis* (Smith, 1911), based on morphological, biochemical and molecular methods. Its pathogenicity to *T. fluminensis* was confirmed, and a host-range test was performed. Unfortunately, results indicated that the bacterium is not sufficiently host-specific for classical introductions. Observations of the damage caused by fungal pathogens in the field suggest that those with the best potential as biological control agents are *Uredo* sp., *K. tradescantiae* and *Mycosphaerella* sp.

***Keywords***: classical biological control, invasive weed, plant disease.

## Introduction

*Tradescantia fluminensis* Vell. (wandering Jew; local name in Brazil—trapoeraba) is one among a series of weed species of world importance belonging to the Commelinaceae. It is native to South America and is particularly abundant along the coast in Southeastern and Southern Brazil where it forms small patches on humid rocky habitats such as along creek margins. It never forms dense extensive populations, and it is not regarded as a weed of importance in Brazil. Conversely, in situations where it was introduced into exotic tropical and subtropical regions of the world, it became a very serious invader of native ecosystems. It is ranked among the most invasive species of Florida (FLEPPC, 2003) and is particularly harmful to forest ecosystems in New Zealand, affecting invertebrate communities (Toft *et al*., 2001; Standish, 2004), hampering natural processes of forest regeneration and nutrient cycling (Standish *et al*., 2001, 2004; Standish, 2002). It has no significant natural enemies (arthropods or pathogens) in New Zealand (Winks *et al*., 2003). Surveys of fungal pathogens of native weeds in Brazil have yielded a plethora of potential biological control agents over the years (Barreto *et al*., 1995. 1999a,b; Barreto and Evans, 1994; Barreto and Torres, 1999; Monteiro *et al*., 2003; Pereira and Barreto, 2000, 2005; Soares and Barreto, 2006; Seixas *et al*., 2007). Two of the fungal pathogens found during these surveys have been introduced from Brazil into other parts of the world, namely: *Prospodium tuberculatum* (Speg.) Arthur for the biological control of *Lantana camara* L. (Barreto *et al*., 2001a; Ellison *et al*., 2006) in Australia and *Colletotrichum gloeosporioides* f.sp. *miconiae* for the biological control of *Miconia calvescens* Schrank and Mart. ex DC. in Hawaii (Barreto *et al*., 2001b). Although Brazil is considered to be the centre of origin of *T. fluminensis*, there is not a single pathogen recorded to be associated with this plant species in this country in the world literature (Table 1). A cooperative research project recently initiated between the Universidade Federal de Viçosa (Brazil) and Manaaki Whenua Landcare Research New

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**Table 1.** Fungal pathogens recorded on *Tradescantia fluminensis* in the literature (Petrak, 1950; Gómez and Kisimova-Horovitz, 1997; Waipara, 2006; Farr *et al*., 2007).

| Fungal species | Country |
|---|---|
| *Alternaria* sp. | USA |
| *Botrytis cinerea* | USA |
| *Cercospora* sp. | USA |
| *Cladochytrium replicatum* | USA |
| *Colletotrichum* sp. | USA |
| *Phakopsora tecta* | Argentina |
| *Pythium* sp. | Hawaii |
| *Rhizoctonia* sp. | USA |
| *Sclerotinia sclerotiorum* | New Zealand |
| *Kordyana tradescantiae* | Ecuador, Costa Rica |

Zealand Ltd. is aimed at surveying and evaluating the native pathobiota associated with *T. fluminensis* in Brazil for potential classical biological control agents. This paper gives a preliminary account of the pathogens found during these surveys and their potential as classical biological control agents for *T. fluminensis*.

## Materials and methods

The field survey was systematic and involved all states of Southern Brazil. Records of *T. fluminensis* were compiled from eight Brazilian herbaria. The following Southern and Southeastern Brazilian states were visited during January 2003 and December 2005: Minas Gerais, Rio de Janeiro, Espírito Santo, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul. Further details of the procedure adopted for the systematic survey can be found in Barreto and Evans (1994). The diseased parts of the plants suspected to be damaged by fungal or bacterial pathogens were collected, dried in a plant press and taken to the laboratory. Seedlings infected by biotrophic fungi were also brought to the laboratory in Viçosa (MG). Fungal structures were removed from specimens and mounted in lactophenol or lactofucsin. Observations of morphology, measurements and illustrations were carried out with an OLYMPUS BX 50 light microscope fitted with a drawing tube. Isolations were conducted by collecting spores from sporulating lesions with a fine pointed needle and plating them on Vegetal Broth Agar medium (Pereira *et al*., 2003). The isolates of non-biotrophic fungi were stored on silica gel according to Dhingra and Sinclair (1995). The materials examined were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC). Additional materials previously deposited at VIC were also examined.

Preliminary pathogenicity experiments were conducted for all the basidiomycetous fungi found, i.e. *Ceratobasidium* sp., *K. tradescantiae* (Pat.) Racib. and *Uredo* sp. *T. fluminensis* plants originating from Brazil or imported from New Zealand (NZ) were used in the pathogenicity experiments. To verify the pathogenicity of *K. tradescantiae*, the fungus was cultivated in Melin-Norkrans modified medium (MNM) and incubated in the dark at 25°C. After 10 days, sporidia were collected by pouring 30 ml of sterile water on the culture surface and scraping it with a rubber spatula. The resulting suspension was filtered through four layers of cheese cloth, and the final concentration of the suspension was adjusted to 1 $10^{7}$ sporidia/ml for inoculation. The cell suspension was sprayed on the leaf surface (abaxially and adaxially) without wounding. After inoculation, 10 plants were covered for 48 h with plastic bags wetted inside and having water-soaked cotton internally and left at room temperature (approximately 25°C). After that period, the plastic bags were removed, and plants were maintained in a greenhouse (26 ± 2°C) and watered daily. Ten non-inoculated healthy plants, kept under the same conditions, served as controls. For the biotrophic fungi *Ceratobasidium* sp. and *Uredo* sp., ten healthy potted *T. fluminensis* plants imported from NZ were cultivated side-by-side (pots kept 5 cm apart) with diseased plants collected during field surveys. Plants were kept for 1 year on a shaded bench outdoors and watered regularly.

## Results

Five fungal species and a bacterium, collected in four different states, were found associated with diseased *T. fluminensis*: three basidiomycetes—a rust fungus (*Uredo* sp.), *K. tradescantiae*—causing white smut-like symptoms and the blight-causing-fungus *Ceratobasidium* sp.; a leaf-spot- and stem necrosis-causing hyphomycete—*Cercospora apii* Fresen. and an ascomycete that is associated with leaf-spots—*Mycosphaerella* sp. (Table 2). The phytopathogenic bacterium was identified as *Burkholderia andropogonis* (Smith, 1911). Three of the fungal pathogens were isolated in culture: *K. tradescantiae*, *C. apii* and *Mycosphaerella* sp. Repeated attempts to isolate *Ceratobasidium* sp. associated with leaf blight were unsuccessful. We believe that this fungus is in fact a biotroph, since often even a complete colonization of the abaxial surface of leaves

**Table 2.** Fungal pathogens found on *Tradescantia fluminensis* during field surveys in Brazil.

| Fungal species | Distribution in Brazilian states[a] |
|---|---|
| *Ceratobasidium* sp. | SC (1); RS (3) |
| *Cercospora apii* | MG (2); SC (1); PR (2) |
| *Mycosphaerella* sp. | RS (1) |
| *Uredo* sp. | PR (1); SC (1); RS (2) |
| *Kordyana tradescantiae* | PR (3); SC (3); RS (9) |

[a] The numbers in parentheses represent how many times each fungus was collected in a state (*MG* Minas Gerais; *PR* Paraná; *RS* Rio Grande do Sul; *SC* Santa Catarina).

(easily observed by an extensive external coverage of the tissues by a mycelial mat) was not accompanied by any sign of necrosis. Attempts to isolate *K. tradescantiae* on vegetable broth-agar (VBA) were unsuccessful. Several other culture media were also tried such as potato-dextrose agar (PDA), corn-meal-agar (CMA), potato-carrot agar (PCA; Dhingra and Sinclair, 1995) but also failed to promote any fungal growth. *K. tradescantiae* was finally successfully isolated on MNM, a culture medium commonly used for ectomycorrhizal basidiomycetous fungi (Marx, 1969).

No disease symptoms were observed on plants from Brazil or NZ inoculated with *K. tradescantiae*, and no symptoms were observed to spread when placing Brazilian plants infected by *Uredo* sp. and *Ceratobasidium* sp. beside healthy NZ plants after over 1 year of observation.

## Discussion

*Kordyana tradescantiae* and *Uredo* sp. are reported for the first time on *T. fluminenis* in Brazil. *K. tradescantiae* has been reported on *T. fluminensis* only from Ecuador (Petrak, 1950) and Costa Rica (Gómez and Kisimova-Horovitz, 1997), and no rusts had been reported on *T. fluminensis* in Brazil (Hennen *et al*. 2005). Although in some field situations these biotrophic basidiomycetes caused no severe disease symptoms on *T. fluminensis*, on other occasions (particularly under heavily shaded areas), damage was significant. Diseased plants appeared weakened and defoliated as compared to healthy *T. fluminensis* plants. It seems that both fungalspecies are promising candidates for the classical biological control of *T. fluminensis* (Table 3). Although *K. tradescantiae* is known to attack plants belonging to several genera in the Commelinaceae, this is not a limitation for its use as a biological control agent in New Zealand where there is not any native plant nor any crop plant of relevance belonging to this family. The other basidiomycete, *Ceratobasidium* sp., caused no significant damage in the field. It still remains unclear whether the slight blight symptoms appearing on colonized leaves only represent naturally senescent leaves or become necrotic because of the fungus infection. This species does not appear to deserve further consideration as a possible candidate agent (Table 3). It is nevertheless interesting to note that no *Ceratobasidium* species has previously been reported on the genus *Tradescantia*, and no other species in this genus was ever reported as a foliar biotroph (Roberts, 1999). Based on the morphological characteristics observed during this study, *Ceratobasidium* sp. was recognized as a new species that will be described separately.

*C. apii* and *Mycosphaerella* sp. caused severe necrotic disease symptoms on leaves and stems of *T. fluminensis* in the field. Crous and Braun (2003) listed numerous hosts belonging to many distinct plant families for *C. apii*; however, this is the first report of *C. apii* on *T. fluminensis*. Despite its supposedly wide host range, we are planning to conduct host range tests based on the centrifugal phylogenetic method (Wapshere, 1974) to evaluate the specificity of this isolate of *C. apii*. It is known that specificity can exist in populations within a fungal species known to have a wide host range (Barreto *et al*., 2001b; Pereira *et al*., 2003). In case this isolate prove to be host-specific, *C. apii* may deserve further consideration for use in classical biological control.

No *Mycosphaerella* spp. was ever reported attacking members of *Tradescantia*. A study of the morphology

**Table 3.** Characteristics of fungal pathogens found on *Tradescantia fluminensis* during field surveys in Brazil.

| Fungal species | Disease | Damage to host | Likely specificity | Cultured | Biological control potential |
|---|---|---|---|---|---|
| *Ceratobasidium* sp. | Faint leaf-blight | insignificant | High | No | Low |
| *Cercospora apii* | Leaf-spot | Significant | Non-specific | Yes | Uncertain |
| *Mycosphaerella* sp. | Leaf-spot | Significant | High | Yes | High |
| *Uredo* sp. | Rust | Significant | High | No | High |
| *Kordyana tradescantiae* | ‘White smut’ | Significant | Low (on Commelinaceae) | Yes | High |

of *Mycosphaerella* sp. from *T. fluminensis* indicates that this is a new species which will be described elsewhere. As it causes severe necrotic disease symptoms on *T. fluminensis* and members of *Mycosphaerella* are often host-specific, this fungus is being considered as a promising candidate for the classical biological control of *T. fluminensis* (Table 3).

The preliminary pathogenicity tests with *K. tradescantiae*, *Uredo* sp. and *Ceratobasidium* sp. were unsuccessful. No disease symptoms were reproduced through artificial inoculation (with *K. tradescantiae*). Perhaps sporidia of this fungus produced in culture are non-infective, and the fungus relies on basidiospores as an infective stage or, perhaps, the fungus loses pathogenicity when cultivated. Nevertheless, it was observed that this disease was naturally transmitted from diseased to healthy plants in Viçosa. This did not happen with either *Uredo* sp. or *Ceratobasidium* sp.

It was observed that plants infected with *Uredo* sp. and *Ceratobasidium* sp., brought from the field in Southern Brazil, were gradually cured of infection along the months after being cultivated under Viçosa conditions at a lower latitude. This suggests that those two fungal species depend on a cooler climate to produce new infection cycles and, therefore, to preserve their populations.

The bacterium collected in the state of Rio de Janeiro was identified as *B. andropogonis*, and its pathogenicity was demonstrated. Inoculated plants of the biotype brought from New Zealand were highly susceptible to this pathogen, and plant death commonly resulted from inoculations. Unfortunately, host-range tests indicated that, although restricted to monocots, this bacterial isolate was capable of infecting all eight species of the Commelinaceae included in the test plus species in five additional families, i.e. pinnaple (Bromeliaceae), *Paepalanthus macrocephalus* (Eriocaulaceae), maize and sorghum (Poaceae), cattail (Typhaceae) and ginger (Zingiberaceae). Although *B. andropogonis* is not known to be a pathogen of maize or sorghum in Brazil, further studies are needed to fully clarify the risk represented by *B. andropogonis* to crop and non-crop plants. Until then, its potential for introduction into New Zealand or other regions of the world or its use as a bioherbicide cannot be considered any further.

Until now, only a limited area of the Neotropics was surveyed for *T. fluminensis* pathogens. It is expected that the continuation of the surveys with the expansion to new areas of natural occurrence of *T. fluminensis* will result in new additions to this still-limited list of pathogens. In addition, the potential for biological control of the pathogens already found is being evaluated. One ongoing study aims at checking the susceptibility of the New Zealand biotype of *T. fluminensis* to the rust and to *K. tradescantiae*. Stations with sentinels (potted plants of the New Zealand biotype) were established at selected places where these pathogens occur on native populations of *T. fluminensis* in Southern Brazil. These will be visited at 3-month intervals for inspection of natural attack by pathogens or arthropods.

Genetic studies are also underway to both clarify the clonal status of *T. fluminensis* in New Zealand as well as compare some DNA regions of the NZ biotype with those found in the native Brazilian range. Chloroplast DNA from over 40 plant specimens from different regions of Brazil are being sequenced to obtain information on the plants' lineages that may help to locate where in Brazil the NZ biotype has originated. These results may then be used to source suitable biotypes of each pathogen agent.

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# Field and laboratory observations of the life history of the Swiss biotype of *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae)

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*Summary*

The Italian biotype of ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae) is considered the most important biological control agent for the suppression of tansy ragwort, *Senecio jacobaea* L. (Asteraceae), in Mediterranean climates. Repeated attempts to introduce this beetle into colder climates have failed to establish populations capable of weed control. The spread of tansy ragwort into the northern Rocky Mountains of Montana prompted a reexamination of the cold-adapted biotype of *L. jacobaeae* reported from Switzerland. A detailed field and laboratory examination of *L. jacobaeae* life history from naturally occurring populations in central Europe was conducted. Adult flea beetles, first collected in late June, started oviposition 2 weeks after emergence from pupae and reached peak oviposition rate after 4 weeks. Over-wintering occurs in the egg stage, as diapause delayed hatch until spring. Larvae initially fed within the leaf tissues in early spring and moved into the root crowns later in the season. Over 70% of second-year plants dissected were infested with larvae in naturally occurring field populations. *L. jacobaeae* from Switzerland were found to be phenologically adapted to continental climates and were released in Montana starting in autumn 2002.

***Keywords***: *Senecio jacobaea*, Montana, ragwort flea beetle.

## Introduction

The ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae), is a specialist herbivore that feeds on the invasive weed tansy ragwort, *Senecio jacobaea* L. (Asteraceae), a biennial herb native to Eurasia (Frick, 1970). *L. jacobaeae* populations originating in Italy are considered the cornerstone of the successful biological control of tansy ragwort in the Pacific Northwest west of the Cascade Mountains (Hawkes and Johnson, 1978; McEvoy and Coombs, 1999). *S. jacobaea* population densities were reduced by 99% in the first 4 years near Fort Bragg, California as a result of root feeding by *L. jacobaeae* larvae (Hawkes and Johnson, 1978). The discovery of a major infestation of tansy ragwort in northwest Montana rekindled interest in the biological control of this noxious weed. The previous success of *L. jacobaeae*, along with its compatibility with other biological control agents released in Montana (Hawkes and Johnson, 1978; Markin, 2003), made introduction of this agent a high priority. However, repeated attempts to establish populations from Oregon into Montana from 1998 to 2002 met with little success (Markin, 2003). Italian *L. jacobaeae* adults collected from Oregon emerge from pupation in the spring, aestivate throughout the summer and await autumn rains to start reproduction and larval development. Adaptations for Mediterranean habitats may limit the ability of the Italian strain to increase populations in colder climates.

This study was initiated to explore the suitability of populations of *L. jacobaeae* from north-western Switzerland for introduction in Montana. Comparisons of the autumn-breeding Italian and summer-breeding Swiss flea beetle strains were conducted under laboratory and greenhouse conditions in Rome, Italy

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(Frick, 1971; Frick and Johnson, 1972, 1973; Windig, 1991). However, over-wintering traits were never field-validated, and the mechanisms of winter survival of the Swiss strain were merely speculated (Windig and Vrieling, 1996). Further, descriptions of egg diapause may have contained experimental artifacts because of interbreeding of strains in the laboratory (Frick and Johnson, 1972). Our first objective was to determine the over-wintering life stage of the Swiss flea beetles in their native habitat and to clarify the patterns of egg diapause under controlled conditions. We then examined the distribution and development of Swiss *L. jacobaeae* under field and greenhouse conditions to determine if their life history traits may better suit this strain to the short summer/cold winters found in Montana's continental climate.

## Materials and methods

Studies on the ecology and seasonal phenology of *L. jacobaeae* were conducted during the summers of 2000 through 2002 at four field sites in Switzerland: L'Himelette, St. Imier, Mervilier, and Mettembert (Table 1). Sites were located on gentle to moderately steep, south to south-western facing slopes in seasonally grazed pastureland. The plant community was dominated by grasses, with mixed forbs occupying less than 35% cover. Tansy ragwort occurred in scattered patches at densities of up to 30 plants per square metre. Snow cover varied between years but extended into mid-March only at the high-elevation L'Himelette site when spring phenologies were observed in 2001. Laboratory experiments were conducted at the Biological Control Containment Facility at Montana State University, Bozeman, MT. After approval for release was granted for the Swiss populations in 2002, further garden and greenhouse experiments were conducted at the USDA Forest Service-Rocky Mountain Research Station in Bozeman, Montana.

### Field observations

Adult emergence from pupation was determined through weekly vacuum samples carried out in Switzerland prior to and during the adult emergence from pupation in June–July 2001. The first field-collected adult ragwort flea beetles were used to determine the starting date of oviposition from different populations. Adults were sexed, and paired beetles were placed in 1-l clear plastic cylinders with fresh-cut *S. jacobaea* leaves inserted in 2-cm cubes of moistened floral foam placed on top of a 90-mm filter paper. Five replicates were used for each population. Every 2 to 3 days, beetles were transferred to clean containers with fresh food, and cylinders, leaves, paper, and foam were inspected for eggs.

The spatial and temporal aspects of *L. jacobaeae* larval biology of Swiss populations were investigated in 2001. Plant samples were initially collected March 15 and then, starting April 15, every 2 weeks from the four sites. The last sampling period was July 27 at the lower elevation sites and August 11 at L'Himelette. For each site, ten samples were collected along a wandering transect starting at a randomly selected point in the pasture. Samples included a tansy ragwort plant and all of the soil and other plants within a 10-cm radius. Samples were removed using a spade and hand trowel and stored individually in a plastic bag at 2°C until dissection.

*S. jacobaeae* plants from each sample were categorized into five demographic groups: seedling (<5 leaves), rosette, multiple rosettes (attached to a single root crown), bolting, and flowering. Plants were dissected, and the locations of larvae and determination of the larval instars were recorded. Larval instars were determined according to the morphology and color of the anal sclerites and the color and width of the head capsule (Newton, 1933; Windig, 1991).

### Laboratory experiments

Two experiments were conducted during the winter of 2002 to 2003 to investigate adult emergence from pupation. The first experiment utilized neonate larvae from St. Imier inoculated on plants maintained in the greenhouse, while the second experiment was conducted at ambient outdoor temperatures with eggs from Mettembert.

Tansy ragwort plants were grown from seeds for 3 months in 3-l plastic pots in the greenhouse with a 14-h photoperiod, and average temperatures of 25°C (day) and 20°C (night). Medium-sized rosettes (average of 17.7 leaves) were randomly assigned *L. jacobaeae* density treatments (0, 5, 10, 15, 20 or 30 eggs or larvae per plant) in a complete block design with five replicates. An additional treatment of five eggs or larvae was initiated as part of each block to be destructively sampled

**Table 1.** First 2001 vacuum collections of adult *Longitarsus jacobaeae* and onset of oviposition for field-collected adults from four field sites in Switzerland.

| Site | Coordinates | Elevation (m) | Adult emergence | Oviposition |
|---|---|---|---|---|
| Mettembert | 47°24′ N, 7°20′ E | 640 | 23 June | 2 July |
| Mervilier | 47°24′ N, 7°18′ E | 660 | 29 June | 4 July |
| St. Imier | 47°09′ N, 6°59′ E | 800 | 7 July | 10 July |
| L'Himelette | 47°08′ N, 7°01′ E | 1160 | 13 July | 17 July |

to determine larval developmental stage. Larvae were transferred directly to plants; while cold-treated eggs (minimum 60 days at 2°C) were transferred to the base of the rosettes on blotter paper and covered with moist peat moss.

Plants for the second experiment were acclimatized for 3 weeks (average 10°C) until the season's first persistent snowfall on December 2, 2002. Plants were moved to prepared garden plots immediately after application of egg treatments, covered with snow, and maintained outdoors until July when larval development was nearing completion.

After the larvae from destructive samples from each experiment reached the late third instar and pre-pupal stages, all test plants were placed in opaque emergence cages. As adult beetles moved into clear collection vials, the date of emergence was recorded. At the end of the experiment, the bags were inspected for adult beetles that had not moved into the vials.

The time required for eclosion of eggs of the Swiss biotype from the St. Imier population was investigated to determine the period of egg diapause. Five replicates of ten apparently viable eggs from a single collection date were placed on filter paper in 90 mm diameter Petri dishes loosely sealed with paper towels. Dishes were kept moist by applying water on the outer portion of the paper towel and stored in plastic bags to maintain humidity.

Treatments varied in length of exposure to cold. The zero day treatment was immediately placed in the growth chamber held at a constant temperature (20.0 ± 0.5°C) with a 14-h photophase. All other treatments were cooled to 2 ± 1°C and stored for 20 to 180 days without light. At 20-day intervals, dishes were removed from cold storage and placed in the growth chamber. Eggs were observed daily for hatch or signs of mortality, and all hatched and dead eggs were removed. Remaining viable eggs were returned to the growth chamber.

Statistical analysis was conducted using Minitab® v. 12 (Minitab Inc., 1998) with $\alpha = 0.05$. One-way analysis of variance (ANOVA) was used to compare plant demographic groups for larval feeding damage and infestation rates. Tukey's pairwise comparisons of demographic groups were analyzed using a family $\alpha = 0.05$ (individual $\alpha = 0.0066$). For both the garden and greenhouse adult emergence experiments, data were pooled across all inoculation levels and tested for normality with the Anderson–Darling normality test. The non-parametric Kruskal–Wallis test was then used to compare egg and larval inoculation treatments for rates of adult emergence. In the egg diapause experiment, the mean egg hatch from each replicate represented samples of the larger population and were normally distributed ($A^2 < 0.603$, $df > 4$, $P > 0.05$). We compared cold treatments using the calculated parameters of each replicate in the one-way ANOVA model. Simple linear regression analysis was used to describe change in eclosion rates. Comparison of regression lines were accomplished using paired *t* tests.

# Results

## Field observations

Adult flea beetles emerged in early summer in the 2001 field surveys in Switzerland (Table 1). The first adult *L. jacobaeae* flea beetles were collected during the third week of June at 600 m sites. Adult emergence from higher elevations was delayed approximately 1 week for every 200 m of increased elevation (Table 1). Newly emerged adults continued to be collected for a period of 2 to 3 weeks at low and medium elevations. Beetles from the highest elevation site emerged over a longer period, possibly due to variation in egg hatch caused by delayed snowmelt in microhabitats shaded by nearby conifer trees. First adult emergence from the St. Imier and L'Himelette sites occurred approximately 120 days after the collections of neonate larvae in March 2001. Our results are at least a month later than those reported by Frick (1971), but beetles transported from Switzerland to Rome, Italy, and Albany, CA, may have started development sooner due to exposure to lower elevations and earlier warm weather.

Adult flea beetles collected in Switzerland began oviposition within 2 weeks of the first adult collections (Table 1). Populations from higher elevations were first collected later in the season and had a corresponding later onset of oviposition. After the initial onset of oviposition for each population, additional collections of adults commenced oviposition immediately following transport to the laboratory.

Larval feeding damage was apparent in 72% of all plants dissected from Swiss field sites in 2001, although larvae were recovered from only 37% of these plants (Figure 1). Larval presence varied significantly among plant demographic groups ($F_{4,395} = 5.40$, $P < 0.001$), with seedlings and flowers having fewer larvae than rosettes, multiple-rosettes and bolting plants (Figure 1). Seedlings comprised 15% of the plants examined but were damaged significantly less than other plants despite their availability in all sampling periods (Figure 1). Only first-instar larvae were recovered from seedlings, possibly because these plants were too small to sustain larval development beyond this stage. Bolting plants occurred later in the season (May 1 at lower elevations) and were predominantly infested with third larval instars. Flowering plants were first collected on June 28 at lower elevations, coinciding with the beginning of adult emergence at these sites (Table 1).

Larvae of the Swiss *L. jacobaeae* had a distinct spatial distribution pattern within the plant that changed over time as the larvae and plants developed (Figure 2). Almost all early-season larvae (94.7%) were found in the basal rosette leaves of *S. jacobaea*. Freshly hatched larvae entered the plant through the leaf blade and fed

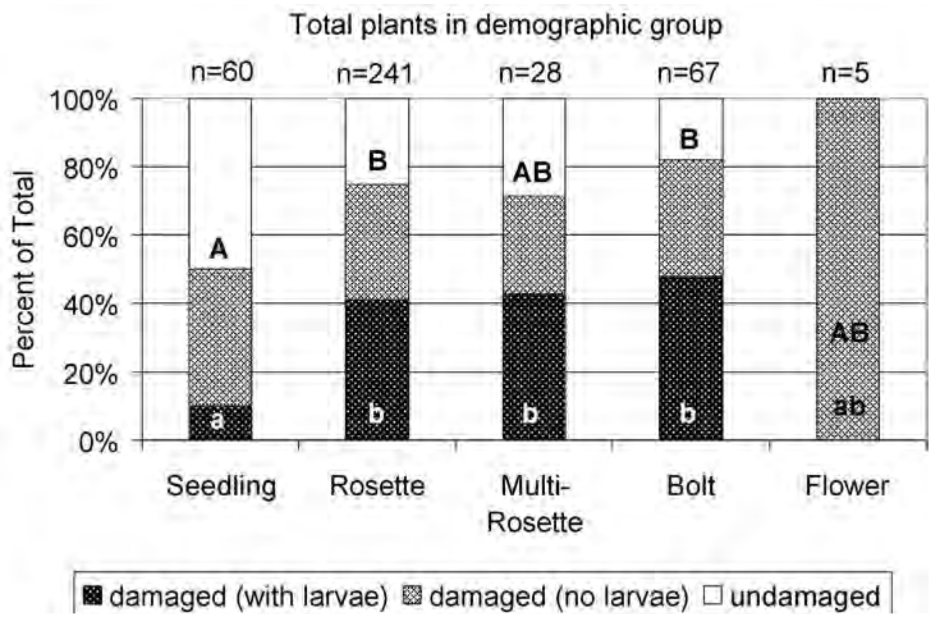


**Figure 1.** Larval utilization of available tansy ragwort plants by plant demographic group by Swiss 2001 field populations of *Longitarsus jacobaeae* (*n* = total). *Shaded portions of bar graphs* indicate feeding damage. *Means with the same letter* (*capital letter*, damage; *lower case*, larval infestation) are statistically indistinguishable in Tukey's pairwise comparisons ($\alpha = 0.05$).

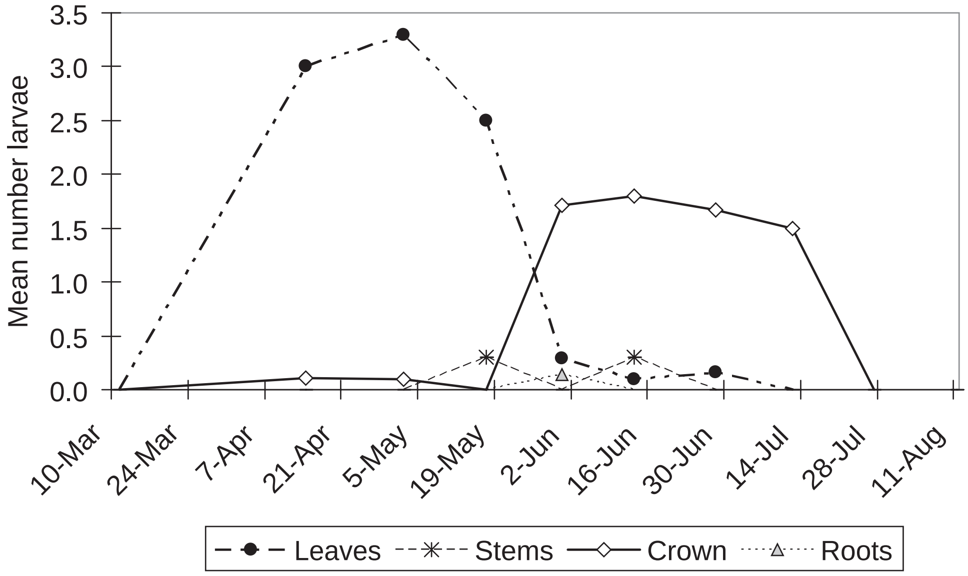


**Figure 2.** Mean number of *Longitarsus jacobaeae* larvae per tansy ragwort plant, separated by plant region, throughout the growing season in the 2001 L'Himelette, Switzerland, population (1200 m).

between the epidermal layers as they moved toward the leaf veins and petiole. Once the first instars reached a leaf vein, they continued to feed downward to the base of the petiole. It was common to find more than one larva in a single leaf, with their feeding tunnels intertwined in the petiole. Later-season larvae moved into the upper root crown (Figure 2). A small percentage (6.1%) of the larvae of all instars was found within the base of stems of bolting plants; however, there were no larvae or signs of larval feeding damage in stems above the lowest stem leaf or approximately 2.5 cm above the basal rosette. The majority of third-instar larvae (83.5%) were found within the root crown. Larval feeding on the root crown occurred in the root cortex. Larvae were rarely collected from the roots (0.43% of total), and most damage to roots occurred only 1 cm away from the root crown as third-instar larvae exited the plant for pupation in late spring.

## Laboratory experiments

*L. jacobaeae* larvae raised under ambient conditions in the garden in Montana completed their development 130.7 ± 4.8 days (*n* = 41) after snowmelt, with mean

adult emergence on July 20, 2003. Adult emergence occurred over a period of 3 weeks (Figure 3), and the time required for emergence did not differ between egg-density treatments (Kruskal–Wallis test, $H = 1.71$, $df = 4$, $P = 0.788$). There were also no significant differences in percent of adult emergence from different-density egg inoculation treatments (Kruskal–Wallis test, $H = 3.90$, $df = 4$, $P = 0.450$). Development rates observed in Montana were slightly longer than the average development rates observed in Switzerland but were well within the range observed at higher elevation sites.

Second-generation St. Imier beetle larvae placed directly on greenhouse plants completed their development in an average of 80.6 ± 0.1 days ($n = 61$) after inoculation. These beetles emerged over a period of 21 days (Figure 3). There was no significant differences in percent of adult emergence among treatments (Kruskal–Wallis test, $H = 1.91$, $df = 4$, $P = 0.752$). The time required for complete larval development corresponded with observations by Frick (1971), who found that laboratory-reared larvae required an average of 80 days to complete development.

The Swiss ragwort flea beetles have been reported to have a facultative egg diapause (Frick, 1971). In our study, eggs of the Swiss biotype hatched after an average of 65.1 ± 2.9 days without exposure to cold temperature when kept at a constant 20°C (Figure 4), but the eclosion period was reduced after exposure to cold temperatures. The time required for eggs to hatch was negatively correlated with time of exposure to cold over the first 60 days ($y = -0.733x + 67.068$, $R^2 = 0.938$, $P \leq 0.001$). Time to eclosion continued to decrease for cold treatments of 80 days or more but at a more gradual rate ($y = -0.0516x + 19.995$, $R^2 = 0.532$, $P < 0.001$). We estimated that diapause was completed after 69 days of cold exposure at the point that the

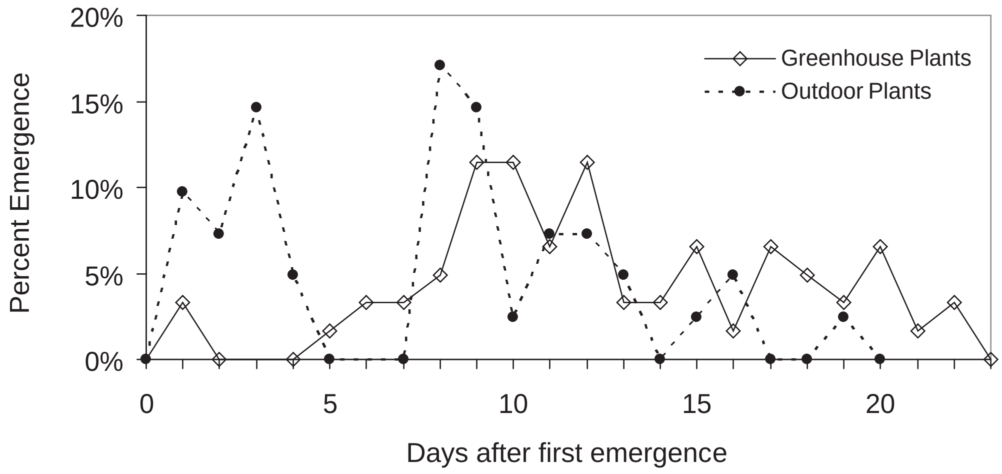


**Figure 3.** Adult *Longitarsus jacobaeae* emergence from the populations raised in the greenhouse (from St. Imier, $\bar{x} = 11.6 \pm 0.8$ days, $n = 61$ adults) and under open-field conditions at Bozeman, MT, in 2003 (from Mettembert, $\bar{x} = 6.7 \pm 0.1$ days, $n = 41$ adults).

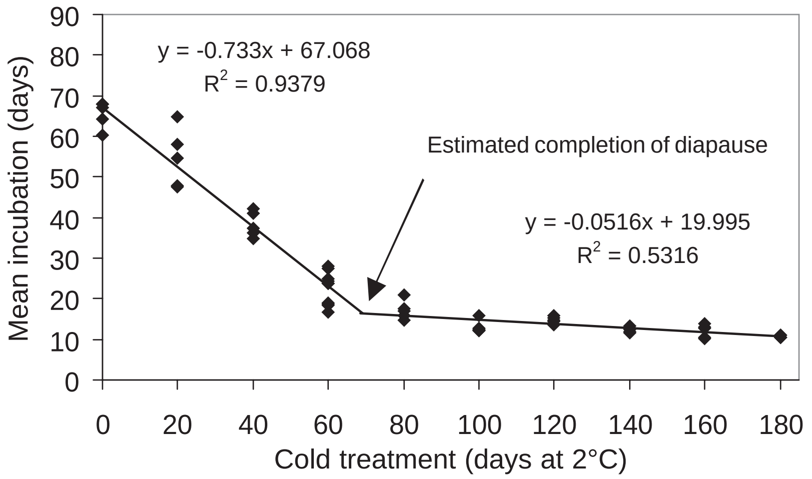


**Figure 4.** Mean incubation period (per replicate) for Swiss *Longitarsus jacobaeae* eggs at 20°C with 14 h photophase after removal from cold treatments (2 ± 2°C). The *regression lines* are significantly different ($t = 17.31$, $df = 46$, $P < 0.0001$).

regression lines intersect (Figure 4; significant change in regression lines: $t = 17.31$, $df = 46$, $P < 0.0001$).

## Discussion

We found that Swiss populations of *L. jacobaeae* have several life history traits that make them suitable candidates for biological control in cold continental climates. Adult beetles are reproductively active throughout the summer. They lay diapause eggs that persist through the winter and are ready to hatch in early spring. The larvae attack the rosette plants just as they are recovering from winter stress. Larvae of Swiss *L. jacobaeae* inhabit fresh leaves in early development and then move into the root crowns during the second and third instars. Damage caused by larval feeding affects plant growth and may reduce reproductive output of their host plant. Large numbers of larvae have been recorded from tansy ragwort in open-field host tests and can cause mortality at high densities (Hawkes, 1968; Puliafico, 2003).

The ragwort flea beetles collected in Switzerland differed in several important life history traits from those originally studied by Frick (1971). No signs of over-wintering larvae or pupae were found during extensive field collections and observations during the 3 years of this study. Larvae of the Swiss populations demonstrated distinct spatial partitioning of their host plants as they develop, with first and early second-instar larvae almost exclusively inhabiting the foliage and above-ground portions of the plant and most third-instar larvae found only in the root crowns. Finally, adult emergence was recorded at the end of June and first 2 weeks of July in their native habitats in Switzerland. Almost all of the differences between our results and those originally published by Frick (1971) can be attributed to laboratory artifacts caused by raising cold-adapted Swiss flea beetles under conditions better suited for their Mediterranean counterparts from Italy. We also found strong evidence of egg diapause broken by extended cold treatment. Crossbreeding of Swiss and Italian strains (Frick and Johnson, 1972) may have contributed to some of the inconsistencies between our egg eclosion data and those previously reported. Much of the confusion caused by these laboratory results has been repeated throughout the subsequent literature (e.g., Hawkes and Johnson, 1978; Windig, 1991, Coombs *et al.*, 1999). Clarification of Swiss *L. jacobaeae* life history traits will improve the ability to establish these beetles for biological control, while increasing our understanding of their potential impact on tansy ragwort infestations in colder continental climates.

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# Fungal survey for biocontrol agents of *Ipomoea carnea* from Brazil

**D.J. Soares and R.W. Barreto**

*Summary*

*Ipomoea carnea* Jacq., also known as morning glory, is native of tropical America, and its purported centre of origin is the Paraguay Basin. This plant is feared by ranchers because of its well-documented toxicity to cattle. Because of its showy flowers, it became a popular ornamental in Brazil and was introduced into others countries, becoming an aggressive wetland ecosystem invader. Little is known about its mycobiota in Brazil which may include fungal pathogens that could be used in classical biocontrol programmes. *Coleosporium ipomoeae* (Schwein.) Burril and *Puccinia puta* H.S. Jacks. and Holw. ex F. Kern, Thurst. and Whetzel are the only fungi recorded in the literature attacking this plant in Brazil. An intensive search for specialized, coevolved fungal pathogens of *I. carnea* was initiated in 2003 in Brazil. Twenty-one fungal species were collected. Among these were the two previously known rusts, *C. ipomoeae* and *P. puta*, and *Aecidium* sp., *Albugo* sp., an unidentified *ascomycete*, *Mycosphaerella* sp., five coelomycetes (*Colletotrichum* sp., *Phoma* sp. *Phomopsis* sp., and two *Phyllosticta* spp.) and ten hyphomycetes (*Alternaria* sp., *Cercospora* sp., *Cladosporium* sp., *Curvularia* sp., *Dactylaria*-like, *Fusarium*-like, *Nigrospora* sp. *Passalora* sp. and two *Pseudocercospora* spp.). Observations of the damage caused by such fungal diseases in the field indicate that the fungi with the best potential as biological agents are *C. ipomoeae, P. puta*, *Albugo* sp., the *Phyllostica* sp. that colonizes stems, and *Phomopsis* sp.

***Keywords***: aquatic weeds, biological control, coevolved pathogens, *Ipomoea fistulosa*, *Ipomoea carnea* subsp. *fistulosa.*

## Introduction

Morning glory, *Ipomoea carnea* Jacq., (local name in Brazil is algodão-bravo) is a shrubby perennial amphibious plant belonging to the Convolvulaceae. It is considered to be native to South America and particularly common in the basins of the rivers Paraguay and São Francisco (Lorenzi, 2000). It is also widely distributed in Brazil as an ornamental species for its showy violet flowers (Kissmann and Groth, 1995). This plant is also one of the most feared poisonous weeds to Brazilian ranchers since it is able to cause severe nervous disorder when ingested by bovines, sheep or goats (Tokarnia *et al*., 2000).

*Ipomoea carnea* was introduced into areas outside the Neotropics, and it now causes serious invasions of wetland habitats in Southern India and Pakistan where streams, mangroves and other ecosystems may be blocked, hampering irrigation and access (H.C. Evans personal communication, 2006). It is also included in the Florida Exotic Pest Council's List of Florida's Most Invasive Species as a weed category II (FLEPPC, 2003).

Surveys of fungal pathogens of plants native to Brazil that are weeds elsewhere have yielded a plethora of potential biocontrol agents (Barreto and Evans, 1994, 1995a,b, 1998; Barreto and Torres, 1999; Barreto *et al*., 1995, 1999a,b, 2000; Pereira and Barreto, 2000, 2005; Monteiro *et al*., 2003; Soares and Barreto, 2006; Soares *et al*., 2006), and two of the fungi highlighted as promising classical biocontrol agents during such surveys have already been introduced from Brazil into other regions of the world: *Prospodium tuberculatum* (Speg.) Arthur for the biological control of *Lantana camara* L. (Ellison *et al*., 2006) in Australia and *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. for the biological control of *Miconia calvescens* DC in Hawaii (Barreto *et al*., 2001).

Recently, a survey for fungal pathogens of *I. carnea* was started, aimed at finding fungi to be used in the future as biocontrol agents for this weed. *Puccinia puta* H. S. Jacks. and Holw. ex F. Kern, Thurst. and Whetzel

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was the only fungus previously recorded on *I. carnea* (referred to as *Ipomoea crassicaulis*) in Brazil. Recently, a second rust fungus, *Coleosporium ipomoeae* (Schwein.) Burril, was recorded by Vieira *et al*., (2004).

## Materials and methods

The collecting procedure adopted during the survey was as described in Barreto (1991). The collecting trips occurred between July 2004 and February 2006. Information on some ad hoc collections that were made before the main survey work is also included. The survey covered a wide geographic area of central and southern Brazil including the states of Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo, Paraná, Santa Catarina, Rio Grande do Sul, Mato Grosso, Mato Grosso do Sul, Goiás and Rondônia.

The diseased parts of the plants suspected to be damaged by fungal pathogens were collected, dried in a plant press and taken to the lab. The isolation of the potential agents was performed by direct transfer of fungal structures to Petri dishes containing 15 ml of VBA medium (Pereira *et al.,* 2003), with the help of a dissecting microscope and a sterilized fine point needle. The fungi obtained were preserved in silica-gel according to Dhingra and Sinclair (1996).

Selected specimens were deposited in the local herbarium (Herbarium VIC). Fungal structures were removed from diseased tissues and mounted in lactophenol. Observations of morphology were carried out with an OLYMPUS BX 50 light microscope.

In order to confirm the pathogenicity of two selected fungi (*Passalora* sp. and *Alternaria* sp.), isolates were cultivated in VBA and incubated in the dark for 48 h at 25°C and later submitted to 12 h near-ultraviolet irradiation and 12 h dark. Four disks taken from 10-day-old cultures were placed abaxially and adaxially on three leaves of two healthy potted *I. carnea* plants. After inoculation, plants were left for 48 h in a humid chamber prepared by covering the plants with plastic

**Table 1.** Fungi recorded on *Ipomoea carnea* from Brazil by Soares (2007).

| Fungus | Disease | Damage to host | Purported specificity | Culturability | Biocontrol potential |
|---|---|---|---|---|---|
| *Aecidium* cf. *distinguendum* | Rust | Significant | To the genus *Ipomoea* | Not cultivable | High |
| *Albugo* sp. | White rust | Significant | High | Not cultivable | High |
| *Alternaria alternata* | Leaf-spot | Significant | Non-specific | Cultivable | Uncertain |
| *Cercospora* sp. | Leaf-spot | Insignificant | Not investigated | Cultivable | Low |
| *Cladosporium* sp. | Associated to leaf-spots | Insignificant | Low | Cultivable | Low |
| *Coleosporium ipomoeae* | Rust | Significant | To the genus *Ipomoea* | Not cultivable | High |
| *Colletotrichum* sp. | Anthracnose (stems) | Moderate | Uncertain | Cultivable | Moderate |
| *Curvularia* sp. | Associated to leaf-spots | Insignificant | Low | Cultivable | None |
| *Dactylaria*-like | Associated to leaf-spots | Insignificant | Uncertain | Cultivable | None |
| *Fusarium*-like | Associated to leaf-spots | Insignificant | Uncertain | Cultivable | None |
| *Mycosphaerella* sp. | Leaf-spot | Moderate | High | Cultivable | Moderate |
| *Nigrospora* sp. | Associated to leaf-spots | Insignificant | Low | Cultivable | None |
| *Passalora* sp. | Leaf-spot | Significant | High | Cultivable | High |
| *Phoma* sp. | Associated to leaf-spots | Insignificant | Low | Cultivable | Low |
| *Phomopsis* sp. | Stem necrosis | Significant | Uncertain | Cultivable | High |
| *Phyllosticta* sp. 1 | Stem and petiole blight | Severe | High | Apparently not cultivable | Very high |
| *Phyllosticta* sp. 2 | Associated to leaf-spots | Insignificant | Uncertain | Cultivable | Low |
| *Pseudocercospora* sp. 1 | Leaf-spot | Moderate | High | Cultivable | Moderate |
| *Pseudocercospora* sp. 2 | Leaf-spot | Moderate | High | Cultivable | Moderate |
| *Puccinia puta* | Rust | Significant | To the genus *Ipomoea* | Not cultivable | High |
| Unidentified Ascomycete | Stem canker | Significant | Uncertain | Attempts unsuccessful | High |

bags wetted inside and having water-soaked cotton internally and left at room temperature (approximately 25°C). After that period, the plastic bags were removed, and plants were left on a bench under room conditions and observed daily for the appearance of symptoms. Three non-inoculated leaves of each of two healthy plants, kept under the same conditions, served as controls.

## Results

Twenty-one fungal species were found in association with *I. carnea* during the survey (Table 1). Among these, at least two taxonomic novelties were promptly recognized and will be dealt with separately in a taxonomic publication, namely: *Passalora* sp. and *Phyllosticta* sp.1. All the other fungi that were found represented new host or geographic records.

Inoculation of *I. carnea* with *Passalora* sp. yielded symptoms equivalent to those observed in the field on all inoculated leaves after 20 days. Non-inoculated leaves remained healthy. Typical structures of the *Passalora* sp. were present on the diseased tissues, and the fungus was re-isolated from newly infected tissues.

The species of *Alternaria* on *I. carnea* had the morphology and cultural characteristics typical of *Alternaria alternata* (Fr.) Keissler. Its pathogenicity to *I. carnea* was proven, and similar symptoms to those observed in the field were observed within 15 days of inoculation. This fungus has not been recorded on *I. carnea* until now.

Attempts to isolate the *Phyllostica* sp.1 associated with stem and petiole lesions were unsuccessful. This fungus appears to have a biotrophic habit. Plant tissues surrounding the fungus colonies were observed to retain a healthy appearance until late stages of infection. Necrosis, leaf drop and death of the apical buds only occurred at the final stages of infection.

## Discussion

At the present stage of this research, it would be too early to dismiss any of the fungi as not promising for use as biocontrol agents for *I. carnea*. Some of the fungi collected in association with *I. carnea* are either evident saprophytes or suspected to have such status, as is the case of *Nigrospora* sp., *Cladosporium* sp., *Curvularia* sp., the *Dactylaria*-like fungus, the *Fusarium*-like fungus and *Phyllosticta* sp.2. Otherwise, the damage associated with the other fungi, listed in Table 1, was significant as observed in the field. In general, plants infected with such fungi appeared weaker and defoliated as compared with individuals in healthy *I. carnea* populations. The fungi appearing to be the most promising candidates for use in weed biocontrol, deserving further evaluations are: the rusts *C. ipomoeae* and *P. puta*, *Albugo* sp., *Passalora* sp., *Phyllostica* sp.1 and *Phomopsis* sp.

Both rust fungi were frequently found throughout the year associated with moderately high plant defoliation. However, they appear to have a wide host range within the Convolvulaceae since both have been recorded on other species in this family, including sweet potato (in the case of *C. ipomoeae*; Hennen *et al*., 2005). There may be host-specific strains of *C. ipomoeae* and *P. puta* that could safely be introduced into other regions of the globe, but even if these species are proven to be polyphagous within the Convolvulaceae, their introduction into other areas of Brazil against noxious *I. carnea* population might still be considered.

*Albugo* sp. was found only a few times, in the states of Mato Grosso, Mato Grosso do Sul and São Paulo. This fungus appears to have a more restricted geographic distribution compared with the two rusts. It caused a complete leaf curling or leaf blight (when the attack occurred on the petioles). However, its specificity and potential to be used as a biocontrol agent requires further investigation.

*Passalora* sp. could prove useful as a classical biological control or even as a mycoherbicide against *I. carnea*. Although no sporulation was obtained for this fungus in the conditions that were used, the potential for mass production of spores, which is critical for its viability as a mycoherbicide, was not properly investigated.

*Phomopsis* sp. was consistently found associated with stem necrosis and easily sporuled in culture; however, its pathogenicty and specificity has not yet been tested.

*Phyllosticta* sp.1 appears to be the most promising candidate to be used as a classical biological control agent. The damage inflicted naturally by this fungus on *I. carnea* populations was evident. Infected plants in advanced disease stages were weakened and almost completely defoliated. On diseased plants, foliage on each individual stem was often reduced to only six or eight terminal leaves.

Although it used to be thought that *A. alternata* had several pathotypes that produce host-specific toxins, this was considered wrong by Simmons (1999). If further investigation on this fungus on *I. carnea* confirms that it fits within the non-specific, cosmopolitan *A. alternata*-group, this would restrict its potential as a classical biocontrol agent but not necessarily result in its rejection for use as a biocontrol agent of *I. carnea*. This fungus grows well and sporulates abundantly in culture and could be further evaluated for development of a mycoherbicide to be used in Brazil similarly to what is being done with an isolate of *A. alternata* obtained from *Eichhornia crassipes* in India (Babu *et al*., 2002, 2003, 2004).

Half of the fungi previously recorded in the literature in association with *I. carnea* were recorded only from countries outside the native range of this plant species in the Neotropics. Most of the records from countries such as India, Pakistan and Malaysia probably represent saprophytic, weakly pathogenic–opportunistic or generalistic pathogens of no relevance for biocontrol (Table 2).

**Table 2.** Fungi recorded on *Ipomoea carnea* and their synonyms worldwide. Extracted from Farr *et al*. (no date).

| Fungus name | Country/Region |
|---|---|
| *Aecidium agnesiae* (Syd.) Z. Urb. | Cuba |
| *Aecidium distinguendum* P. Syd. and Syd. | Caribbean; Cuba; Venezuela |
| *Aecidium* sp. | Venezuela |
| *Albugo ipomoeae* (as spelt by the author) | Cuba |
| *Albugo ipomoeae-panduratae* (Schwein.) Swingle | Caribbean; Cuba |
| *Aplosporella ipomoeae* S. Ahmad | India; Pakistan |
| *Botryodiplodia theobromae* Pat. | Pakistan |
| *Capnodium* sp. | Caribbean; Cuba |
| *Cercospora ipomoeae* G. Winter | India |
| *Coleosporium ipomoeae* | Cuba; Colombia; Brazil |
| *Cytospora ipomoeae* S. Ahmad and Arshad | India |
| *Dischloridium cylindrospermum* S.K. Srivast. | India |
| *Dothiorella ipomoeae* S. Ahmad | India |
| *Guignardia cytisi* (Fuckel) Arx and E. Müll. | Pakistan |
| *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. | Venezuela |
| *Leptosphaeria macrospora* (Fuckel) Thüm. | Pakistan |
| *Macrophoma ipomoeae* Pass.; | India; Pakistan |
| *Marasmiellus scandens* (Massee) Dennis and D.A. Reid | Malaysia |
| *Meliola malacotricha* Speg. | Malaysia |
| *Monilochaetes infuscans* Harter | India |
| *Munkovalsaria donacina* (Niessl) Aptroot | India |
| *Ophiobolus herpotrichus* (Fr.) Sacc. | Pakistan |
| *Periconia byssoides* Pers. | Venezuela |
| *Pestalotiopsis adusta* (Ellis and Everh.) Steyaert | India |
| *Phoma herbarum* var. *herbarum* Westend. | Pakistan |
| *Phomopsis ipomoeae* Petr. | Venezuela |
| *Pseudocercosporella ipomoeae* Sawada ex Deighton | Venezuela |
| *Puccinia achyroclines* (Henn.) H.S. Jacks. and Holw.[a] | Brazil |
| *Puccinia distinguenda* H.S. Jacks. and Holw. | Ecuador; Venezuela |
| *Puccinia megalospora* (Orton) Arthur and J.R. Johnst. | Mexico |
| *Puccinia nocticolor* Holw. | Guatemala |
| *Puccinia puta* | Colombia; Venezuela; Puerto Rico; Brazil |
| *Puccinia rubicunda* Holw. | Mexico |
| *Tuberculina persicina* (Ditmar) Sacc. | Caribbean |

[a] This record is regarded here as dubious, since the original publication (Hennen *et al*., 1982) which was cited by Farr *et al*. (no date) makes no mention of *I. carnea* or its synonyms as host for this fungus.

Although only a relatively limited area of the native range of *I. carnea* was surveyed, several potential biocontrol agents were found. It is, therefore, expected that the expansion of the survey into new areas in Brazil or other parts of the Neotropics will reveal a much larger list of potential fungal agents for biocontrol of *I. carnea*. Although Brazil is considered as part of the centre of origin of this plant, only two fungi were previously known on this host in Brazil. Results of the present study added 19 new taxa to this list, most of which are clearly pathogenic to *I. carnea* (Table 1). Pathogenicity and host-specific tests are now being conducted to confirm the status of fungi selected as having possible potential for use in the biocontrol of *I. carnea.*

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# Biological control of lippia (*Phyla canescens*): surveys for the plant and its natural enemies in Argentina

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*Summary*

Lippia, *Phyla canescens* (Kunth) Greene (Verbenaceae) is a fast-growing, mat-forming plant native to South America. It is a weed in Australia, where it was introduced as an ornamental during the nineteenth century. The knowledge about the biology of lippia is currently limited to unconcluded taxonomical studies; there is scarce information about the ecology and natural enemies in the native range. Surveys for the plant and its natural enemies were initiated in Argentina in 2005 to determine its distribution and to search for possible biological control agents, both insects and phytopathogens. We have found *Phyla* sp. in 54 out of 102 sites sampled, mostly east of 66°W, circumscribing the weed to the Chaco Domain. In places where the plant was present, at least 20 arthropods and 16 fungi were found. Among insects, the most promising candidates are three flea beetles (Chrysomelidae): two species of *Longitarsus* and *Kuschelina bergi* Harold. Pathogens include the rust *Puccinia cf. lantanae* Farl., *Cercospora cf. lippiae* Ellis and Everh. and three *Colletotrichum* spp., associated with leaf spots and stem cankers. Additional information on their biology and host specificity is required to propose any of these as biological control candidates.

***Keywords***: plant distribution, arthropods, pathogenic fungi.

## Introduction

Lippia, *Phyla canescens* (Kunth) Greene (Verbenaceae) is a fast-growing, mat-forming plant. It is widespread in and thought to be native to South America (from southern Ecuador, throughout Peru, Chile, Argentina, Uruguay, Paraguay, Bolivia and Brazil) (Collantes, *et al*., 1998; Múlgura de Romero *et al*., 2003). It was also recorded from fossil pollen in Santa Fe and Buenos Aires Provinces, in Argentina (Alzugaray *et al*., 2003; Fontana, 2005), reinforcing this area as a centre of origin.

*P. canescens* has been reported naturalized from France, Spain, Italy, Algeria, Botswana, Senegal, Egypt, South Africa, New Zealand and Australia (Kennedy, 1992). This plant is a weed in Australia, where it was introduced as an ornamental plant during the second half of the nineteenth century. It is a major threat to biodiversity and riparian areas and has a significant impact on conservation and grazing systems due to its increasing density and distribution (Julien *et al*., 2004), causing annual losses of cattle production of 38 million Australian dollars and even greater estimated losses of environmental service. Current short-term and unsustainable control methods include the use of herbicides, cultivation and grazing management (Earl, 2003; Julien *et al*, 2004). Biological control is proposed as the sustainable method for this weed and may be the only option for conservation areas, woodlands, forests and along stream banks. However, until this study, there was little information about the plant and its natural enemies in the native range.

Worldwide, Kennedy (1992) recognized nine species of *Phyla* including *P. canescens*. In Argentina, an additional species, *Phyla reptans* (Kunth) Greene was recognized together with *Phyla betulaefolia* Greene and *P. canescens* (Múlgura de Romero *et al*., 2003).

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Australia has *P. canescens* and *Phyla nodiflora* Greene (Munir, 1993); however, there is confusion between these two species in the literature, and it remains uncertain whether *P. nodiflora* is native to Australia or not (Leigh and Walton, 2004).

There are no records of arthropods associated with *Phyla* spp. and few for fungal pathogens. In North America, two biotrophic fungi: *Oidium* sp. (powdery mildew) and *Meliola lippiae* Maubl. (black mildew) and the following necrotrophs: *Cercospora lippiae* Ellis and Everh. (leaf spot), *Sphaceloma lippiae* Baines and Cummins (anthracnose) and *Sclerotium rolfsii* Sacc. (southern blight) were identified on *P. nodiflora* and/or *Phyla lanceolata* (Michaux) Greene (Farr *et al*., 1989). *C. lippiae* (syn. *Pseudocercospora lippiae*) occurred on *Phyla strigulosa* (Mart. and Gal.) Moldenke and *Phyla* spp. in the Caribbean, Asia and Africa (Ellis, 1976). On *P. nodiflora* (=*Lippia nodiflora*, including also *P. reptans*?) in India, *C. apii* Fresen. emend. Crous and U. Braun was recorded (Crous and Braun, 2003) and in South America, *Meliola lantanae* Syd. and P. Syd. and *Phoma zappaniae* Speg. (Viégas, 1961). In Argentina, the rust *Puccinia lantanae* Farl. was found on *P. canescens* in Salta Province, on *Phyla* sp. in Tucumán Province and in Paraguay (Viégas, 1961; Lindquist, 1982). There are also records of *Prospodium* spp. on *Lippia* (including *Phyla*) in South America (Viégas, 1961).

To gain knowledge of the natural distribution, centre of origin and natural enemies of *P. canescens* in South America, surveys were initiated in December 2005. The aim was to identify suitable arthropod and phytopathogen biological control agents for Australia.

# Methods and materials

## Surveys

*P. canescens* is recorded in 17 provinces of Argentina (Múlgura de Romero *et al*., 2003). Considering these records and bibliographical information, surveys were conducted in four ecological regions of Argentina from December 2005 to February 2007 (Figure 1): (1) Wetland Chaco, a humid area with a rainy season in summer, characterized as wetlands with patches of mesquite forests, which includes the north-eastern provinces of Entre Ríos, Santa Fe, Corrientes, Chaco and Formosa; (2) Dry Chaco (similar to the wet Chaco but mostly grassland) and Yungas (subtropical mountain rain forest), including the north-western provinces of Jujuy, Salta, Santiago del Estero, Tucumán, Catamarca, La Rioja, Córdoba, Mendoza and San Luis; (3) Pampas, grassland of Buenos Aires Province; and (4) Transition zone between Southern Chaco, Pampas and Patagonia, a cold and dry area including Río Negro, Neuquén and Mendoza but wet in the Río Negro basin where *P. canescens* was found.

*P. canescens* is a small, prostrate plant, often growing as an understory plant and therefore difficult to see. Sampling was conducted along roadsides every 100–180 km. At each site, the presence of the plant was checked, and if present, natural enemies were collected. In 27 sites where plants were difficult to identify in the field (because of absence of inflorescences), specimens were collected and cultivated in the greenhouse for identification. Repeated sampling was conducted at two (region 4) and ten sites (regions 1–3) in each region at different times of the year. Soil was sampled at 14 sites with and without lippia, and pH and relative humidity were recorded. Samples were analysed for phosphates, nitrates, nitrites, ammonia, calcium, phosphorous, iron and humus (LaMotte Combination Soil Model STH-14). This information was examined with Reciprocal Averaging (PC-ord 4).

## Natural enemies

**Arthropods:** Arthropods were collected directly from plants using aspirators or from plant parts attacked by endophagous species (e.g. miners or gall formers) and taken to the laboratory for rearing. Material was placed in plastic containers (8 cm diameter, 5 cm high) with moistened tissue paper and leaves of the plant as food, and kept in growth chambers at 25°C and 12 h light. All adults that were collected or reared were sent to taxonomists for identification. Relevant biological information was recorded.

Laboratory studies were conducted for the flea beetle collected in two places: Tres Arroyos (38.52°S, 60.51°W) and Nueva Atlantis (36.85°S, 56.69°W) in Buenos Aires Province. Eggs laid by field-collected adults were kept in plastic containers (10 cm diameter, 2 cm high), and *P. canescens* leaves were added to the resulting larvae. The last instars were reared in a bigger container, and when the larvae decreased their activity (prepupal stage), they were transferred to another container (8 cm diameter, 5 cm high) with soil as substrate.

**Pathogens:** Plants were inspected for symptoms of disease, representative samples were collected, and the presence of fungi was checked in the lab. When necessary, samples were placed into humid chambers to encourage sporulation. Isolations were made by placing disinfected leaf and stem pieces onto different agar culture media: potato dextrose agar (PDA), water agar (WA) and specific media (*Phyla* leaf-oat meal agar—a modification of carrot leaf-oat meal agar; Dhingra and Sinclair, 1985). The mycelial cultures were exposed to an UV regime for sporulation. Fungi were then identified. The isolated fungi are maintained on PDA. Desiccated specimens of infected plants have been incorporated in the herbarium of the Phytopathology Laboratory of the University of Bahía Blanca.

# Results

## Surveys

*Phyla* sp. was recorded in 54 of 102 sites sampled, mostly east of 66°W and north of 40°S, from sea level (Buenos Aires Province) to 2100 m (Volcán, Jujuy Province) suggesting a natural distribution in the Chaco Region and in the Pampas (Chaco Domain) (Cabrera and Willink, 1980; Figure 1). In the northern half of region 2, pure and mixed stands of *P. canescens* and *P. reptans* and some possibly intermediate forms were found. High phenotypic variation and poor distinguishing characters (presence or absence of conspicuous secondary leaf venation) made it difficult to discriminate these species in the field. Specimens that were taken for identification turned out to be both *P. reptans* and *P. canescens*. Elsewhere, populations found were easily identified as *P. canescens*.

Preliminary analyses of soil samples did not detect any obvious differences between sites with or without *Phyla* spp.

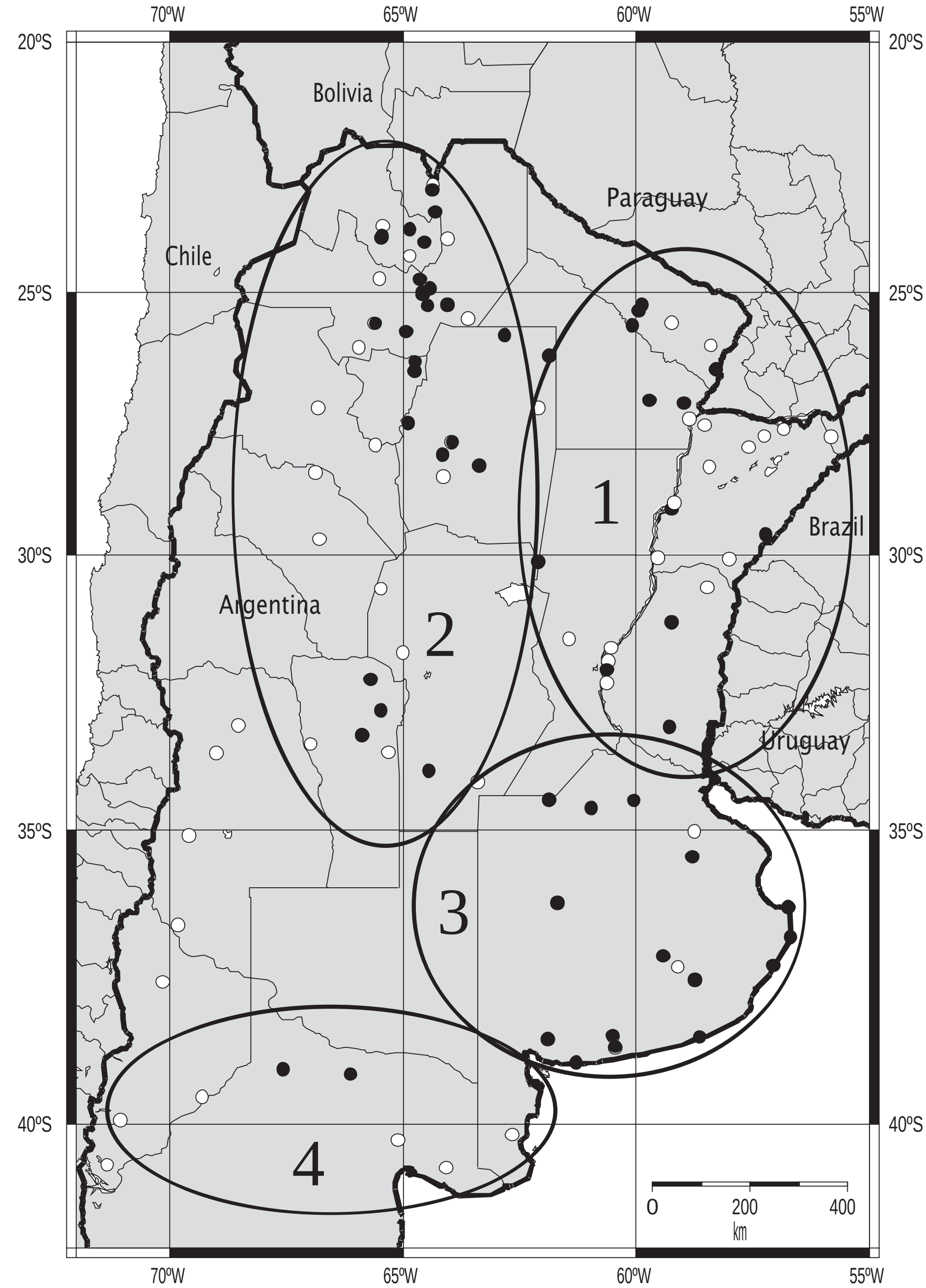


**Figure 1.** Sites sampled for *Phyla* spp. and natural enemies associated. *Black dots* indicate plant presence and *white dots*, plant absence. *1* Wetland Chaco, *2* Dry Chaco and Yungas, *3* Pampas and *4* Transition between Southern Chaco, Pampas and Patagonia.

## Natural enemies

**Arthropods:** So far, about 20 species of arthropods have been found: four flea beetles [*Kuschelina bergi* Harold, *Longitarsus* spp. and *Disonycha glabrata* (Fabricius) (Chrysomelidae)], a leaf mining fly (Agromyzidae?), two thrips (Thysanoptera), four species of Lepidoptera (two micro moths and two hairy caterpillars), eriophyid mites, unidentified stem gallers, four leafhoppers (Cicadellidae) and two Cercopidae. The latter six sap feeders are generalists. Leafhoppers were found throughout the range of both plant species (*P. canescens* and *P. reptans*). Different beetle species had different ranges, sometimes with overlapping distribution; however, *Longitarsus* spp. were found throughout the ranges of both plants. In the field, *Longitarsus* spp. were found only as adults feeding on leaves; attempts to lab rear have not yet been successful.

The flea-beetle collected and studied in the laboratory, *K. bergi*, was only found on *P. canescens* in the Pampas and in the transition zone (regions 3 and 4, Figure 1). It was only found on litter or the ground amongst prostrate stems. In the lab, it has five larval instars and takes about 2 months to complete its life cycle. Pupation occurs in the substrate and takes about 2 weeks. Further studies including host range tests are planned. There is no biological information available on the other natural enemies.

**Pathogens:** At this early stage of the research, at least 16 species of fungi have been found associated with the two *Phyla* spp. Several of them are secondary invaders (e.g. *Nigrospora*, *Sordaria*, *Podospora*), others are clearly pathogenic (*Puccinia cf. lantanae*, *C. lippiae*, *Colletotrichum* spp.), and others might or might not be involved in the etiology of diseases (*Fusarium* sp., *Bipolaris* sp., *Alternaria* sp., *Phoma* sp., *Phomopsis* sp.).

*P. cf. lantanae* was found on *Phyla cf. reptans* in one place in the north-eastern province of Jujuy. Only telia were present on leaves and stems. Teliospores were mainly one-celled, but there were also some two-celled spores. *C. lippiae* was isolated from *P. canescens* associated with stromata on necrotic leaves and circular leaf spots. There are at least three *Colletotrichum* spp. involved in leaf-spot symptoms: *Colletotrichum dematium* (Pers.: Fr.) Grove on *P. reptans* and *Colletotrichum cf. orbiculare* on *P. canescens*, while a third species, tentatively placed in *Colletotrichum*, was found on *P. cf. reptans*.

# Discussion

The relatively short period of surveys so far has provided a range of arthropod and phytopathogen species that are being considered as potential agents. The flea-beetle *K. bergi*, a leaf-feeder, is the first insect to be successfully reared in the lab and will soon undergo preliminary host testing. Other beetles, thrips and Lepidoptera await further observations and studies. The microcyclic rust (*P. cf. lantanae*) is also under consideration, though preliminary studies suggest its host is *Phyla reptans*, the species we collected it from, rather than *P. canescens*, the host recorded by Spegazzini (1909). *C. lippiae* is the most widespread phytopathogen, causing necrotic leaf spots. Three *Colletotrichum* spp. have been isolated and are of interest. We have not yet identified any arthropod–pathogen associations.

The native range of *P. canescens* is thought to be in South America (Kennedy, 1992), although at present the centre of diversity is not known. It is widely dispersed in the Pampas and dry Chaco in Argentina, and this is where surveys have been concentrated so far. Only two of the three *Phyla* species recorded from Argentina have been found. *Phyla reptans*, and possible intermediate forms with *P. canescens*, occur in the wetter north-western areas of Argentina, whereas *P. canescens* appears to be the only *Phyla* species that occurs in the drier Chaco and the vast grassland areas. Recent molecular studies indicate that *P. canescens* might be restricted to the south and that the species in the north is *P. reptans* (M. Fatemi, unpublished data, 2007). Therefore, we tentatively hypothesize that the centre of dispersion of the genus in Argentina is the Chaco Domain.

Future surveys will seek *Phyla* species and their natural enemies in countries neighbouring Argentina. In addition, taxonomic (including morphometrics and molecular analyses) and cytogenetic studies of *Phyla* and repeated collections of natural enemies will help us determine the centres of origin, delimit the native ranges and select potential biological control agents for *P. canescens*.

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# Potential biological control agents of field bindweed, common teasel and field dodder from Slovakia

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*Summary*

Field explorations during 2001 to 2006 in Slovakia resulted in the discovery of several potential biological control agents of the three weeds, field bindweed, *Convolvulus arvensis* L.; common teasel, *Dipsacus fullonum* L.; and field dodder, *Cuscuta campestris* Yuncker. The five top candidates are described in the following. The larvae of the agromyzid fly *Melanagromyza albocilia* Hendel (Agromyzidae) mine in the stems and root crowns of field bindweed, causing the death of infested shoots. The number of infested plants ranged from 46.7% to 99.2% and the number of infested stems from 4.1% to 37.2% in southwest Slovakia. The larvae and adults of the tortoise beetle, *Hypocassida subferruginea* (Schrank) (Chrysomelidae), almost completely destroyed leaves of field bindweed in some uncultivated habitats in the warmest localities of Slovakia. Development of the species is rapid under favourable conditions and takes only 22 to 27 days; females have a high fecundity, and it is easy to rear. The most important natural enemy of *H. subferruginea* recorded in Slovakia was the egg parasitoid *Brachista pungens* (Mayr) (Trichogrammatidae). Adult moths of *Endothenia gentianaeana* (Hübner) and *Cochylis roseana* (Haworth) (Tortricidae) were reared in high numbers from flowerheads of common teasel during the study. Of *E. gentianaeana*, only one larva was found per flowerhead, feeding within the central cavity, while larvae of *C. roseana* were gregarious. Especially *C. roseana* was destroying a large number of seeds within the flowerheads of teasel. Considerable parasitization of *E. gentianaeana* by *Glypta mensurator* (Fabricius) (Ichneumonidae) was noted. Weevils from the genus *Smicronyx* (Curculionidae) were found to be the principal natural enemies of dodders in Slovakia. Larvae of *Smicronyx* spp. induce stem galls, which prevents flowering and fruiting of field dodder vines. *Smicronyx jungermanniae* (Reich) was the most abundant species, accounting for up to 96% of the total number of weevils ($n$ = 877) reared from field dodder galls.

***Keywords***: *Convolvulus*, *Dipsacus*, *Cuscuta*, insect, candidates.

## Introduction

Field bindweed, *Convolvulus arvensis* L. (Convolvulaceae), has been described as the 12th worst weed in the world, the seventh most important in Europe and the most important weed in European orchards. Field bindweed tolerates a great range of environmental conditions and elevations; for more information on bindweed, see Tóth (2000). Although a relatively large number of species has been recorded from the Convolvulaceae, only a small number of them seem to have potential as biological control agents (Tóth and Cagáň, 2005). Of these, only *Tyta luctuosa* (Denis and Schiffermuller) (Lepidoptera: Noctuidae) and *Aceria malherbae* Nuzzaci (Acari: Eriophyidae) have so far been used in the classical biological control of bindweeds in North America (Rees and Rosenthal, 1996).

Common teasel, *Dipsacus fullonum* L. (Dipsacaceae), grows mostly in non crop areas in Slovakia. River banks, roadsides and disturbed areas are the most common habitats of teasel throughout Slovakia. Teasel is an invasive species in North America. Whereas the plant is not a problem on Slovakian agricultural land, it is considered a noxious weed locally in the USA. Teasels are invading plains, waste grounds, old fields, pastures and grow along edges of forests (Sforza, 2002). While a large number of insects feed on teasels, no biological control agent has up to now been introduced.

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Field dodder, *Cuscuta campestris* Yuncker (Cuscutaceae), is an annual stem parasite with leafless, thread-like, orange or yellow stems that twine over other plants. Field dodder is distributed worldwide and has very low host specificity, attacking many different host plants simultaneously. There are five dodder species known in Slovakia, but *C. campestris* is the only introduced species; for more information on dodder, see Tóth and Cagáň (2001). *C. campestris* was introduced from North America to Europe in 1883 (Jehlík, 1998). It is not yet possible to provide an authoritative assessment of the biological control prospects for dodder. The surveys that have been made give an indication of potential biological control candidates, but knowledge of their host range and of the conditions required for them to achieve effective suppression is incomplete (CAB, 1987).

The main objective of this study was to determine the most important insect guild feeding on the above-mentioned weeds in Slovakia and to evaluate their potential use in classical or inundative biological control programmes.

# Materials and methods

## Field bindweed

During the growing season of 1998 to 1999, 2002 to 2003 and 2005, three sites of field bindweed (Kamenica nad Hronom, Čajkov, Vráble) in southwest Slovakia were checked weekly from mid-April until the beginning of October, following the natural phenology of the plant. An additional seven locations were visited three times at monthly intervals. The locations were chosen in different geographic and climatic regions. Besides, opportunistic sampling was conducted at numerous locations with different climates during different times of the year. Collection sites were grassy or weedy roadsides, fallow fields, *C. arvensis*-infested cropland and vacant town lots. At each collection site, plants were inspected for damage. Insects were collected by sweeping (150 sweepings per site), or by aspirating or hand-picking them from plants. The field surveys were concentrated on the agromyzid fly, *Melanagromyza abocilia* Hendel (Diptera: Agromyzidae) and tortoise beetles of the genus *Hypocassida* (Coleoptera: Chrysomelidae). More detailed methods are described in Tóth *et al*. (2005) and Tóth and Tóthova (2006).

Preliminary host-specificity tests were conducted with *M. abocilia*. Two economically important field bindweed relatives in the Convolvulaceae, *Ipomoea batatas* (L.) Lam. (sweet potato) and *Ipomoea alba* L. (ornamental plant) were used in the experiments. Ten potted field bindweed plants were exposed together with ten plants of *I. batatas* and ten plants of *I. alba* at each of three corn fields infested by field bindweed in June 2002 and 2003. At the end of September, plants exposed as well as 30 naturally growing bindweed plants were evaluated for attack of *Melanagromyza albocilia*.

## Common teasel

During 2003 to 2004, the study was extended to plants from the genus *Dipsacus*. Several surveys were conducted from April to June in southwest Slovakia, concentrating on flower-feeding insects. The plant was mostly found in natural areas. To rear adult insects and their parasitoids, flowerheads of *D. fullonum* were collected and placed in glass boxes with a perforated top under lab conditions (20°C, 70% RH). A total of 200 flowerheads was collected. Emerged adults were identified.

## Field dodder

During the growing season 2001, 2003 and 2006, the occurrence of insects feeding on field dodder was observed irregularly in the agroecosystems of Slovakia following the natural phenology of dodders. A total of 82 localities were chosen in different geographic and climatic regions throughout Slovakia. Collection sites were field dodder-infested croplands planted with various crops, fallow fields and roadsides. At each locality, *Cuscuta* species were identified. The localities were inspected especially to record the presence of the weevils from the genus *Smicronyx* (Coleoptera: Curculionidae). At each collection site, field dodder plants were inspected for the presence of stem galls and galls collected. To assess adult emergence, field-collected stem galls were placed in plastic tubes (8 cm diameter, 4.5 cm high) with perforated tops for aeration and kept in the laboratory at 20°C ± 1°C. Emerged adults were identified.

# Results

During the study, 108 organisms were collected from field bindweed (see Tóth and Cagáň, 2005 for details), seven from teasel, and six from dodder.

## Field bindweed

*M. albocilia* Hendel was the only species feeding within the stems and roots of field bindweed in Slovakia. Between 10% and 100% of field bindweed plants and up to 50% of the shoots were infested. The life history of *M. albocilia* and impact on the host were described in detail by Tóth *et al*. (2005). *M. albocilia* was found in 91 locations of 132, confirming it is a common insect in Slovakia and closely related to its host plant *C. arvensis*. Seven species of Hymenoptera were reared from pupae and larvae of *M. albocilia* as solitary, larval and pupal parasitoids belonging to four families: *Aneuropria foersteri* Kieffer (Diapriidae), *Sphegigaster truncata* Thomson *Sphegigaster aculeata* (Walker), *Cyrtogaster vulgaris* Walker (Pteromalidae), *Macro-*

*neura* (*Eupelmus*) *vesicularis* (Retzius) (Eupelmidae), *Chorebus cyparissa* (Haliday) and *Bracon picticornis* Wesmael (Braconidae). Parasitoids from the families Braconidae and Pteromalidae predominated, making up 96.3% of parasitoids that emerged. *S. truncata*, *C. cyparissa* and *B. picticornis* were the most abundant parasitoids reared from *M. albocilia*, accounting for 36.3%, 35.6% and 21.8%, respectively, of the total number reared. Preliminary studies conducted in Slovakia revealed that parasitoids suppressed 77% of the stem miner population in the field.

During preliminary host-specificity tests, *M. albocilia* attack was found in both wild and artificially exposed field bindweed plants at the three field localities but no attack on *I. batatas* and *I. alba*.

Tortoise beetles were important field bindweed defoliators. Seven species were found in association with field bindweed in Slovakia. These were *Cassida sanguinosa* Suffrian, *Cassida nebulosa* Linnaeus, *Cassida stigmatica* Suffrian, *Cassida vibex* Linnaeus, *Cassida murraea* Linnaeus, *Cassida viridis* Linnaeus and *Hypocassida subferruginea* (Schrank) (Coleoptera: Chrysomelidae). The most abundant and widespread species was *H. subferruginea*. The species is recorded as specific on field bindweed and *Calystegia sepium* (L.) R. Br. (Convolvulaceae) in Slovakia (Tóth and Tóthová, 2006). Females deposited oval, light-red eggs in small groups of two to five on the leaf surface of field bindweed (mostly on the underside) from mid-April to the end of May. The larvae fed on leaves from mid-May to the beginning of July. The highest number of adults was found between 21 May and 11 June. For more details of the life history of *H. subferruginea*, see Tóth and Tóthová, (2006). The larvae and adults were able to almost completely destroy the leaves of field bindweed plants in some uncultivated habitats. The only common natural enemy of *H. subferruginea* recorded in Slovakia was the egg parasitoid *Brachista pungens* (Mayr) (Hymenoptera: Trichogrammatidae).

## Common teasel

Seven insect species were found associated with common teasel in Slovakia. These were *Macrosiphum rosae* (L.) (Sternorrhyncha: Aphididae), *Metzneria neuropterella* Zeller (Lepidoptera: Gelechidae), *Endothenia gentianaeana* (Hübner), *Diceratura ostrinana* (Guenée), *Cochylis roseana* (Haworth) (Lepidoptera: Tortricidae), *Myelois circumvoluta* (Fourcroy) (syn. *cribrumella*, *cribrella*) and *Homoeosoma nebulellum* (D. and Sch.) (Lepidoptera: Pyralidae). *M. rosae* and *D. ostrinana* were feeding on leaves and rosettes. Immature stages of other moths occupied the flowerheads. Although most of the species were rare and caused minor damage to the host plant, adult moths of *E. gentianaeana* and *C. roseana* were reared in high numbers from *D. fullonum* flowerheads. Infestation of flowerheads by *E. gentianaeana* reached almost 100% everywhere in Slovakia. Larvae were feeding within the central cavity of flowerheads and destroying the seeds. On average, one larva damaged about ten seeds during each attempt to cut an exit hole. Infestation of plants infested by *C. roseana* ranged from 70% to 100%. Larvae of *C. roseana* were found to feed gregariously (7–30 larvae per flowerhead), and destroyed the largest number of seeds. During the study, several parasitoids were recovered from flowerheads. Considerable parasitization was only recorded for *E. gentianaeana*, where *Glypta mensurator* (Fabricius) (Hymenoptera: Ichneumonidae) dominated.

## Field dodder

Species from four orders were regularly found feeding on dodder plants, i.e. aphids (Sternorrhyncha), bugs (Heteroptera), weevils (Coleoptera) and flies (Diptera). Aphids mostly consisted of *Aphis fabae* Scopoli (Aphididae), bugs of *Lygus rugulipennis* Poppius (Miridae), and the diptera were dominated by the stem-mining fly, *Melanagromyza cuscutae* Hering (Agromyzidae) (for details about *M. cuscutae*, see Tóth *et al*., 2004a). All three species were locally common but not harmful for dodders. Weevils from the genus *Smicronyx* (Coleoptera: Curculionidae) were found to be the principal natural enemies of dodders in Slovakia. Larvae of *Smicronyx* spp. caused stem galls on field dodder. Such damage prevents flowering and fruiting of field dodder vines and destruction of flowers and seeds in other *Cuscuta*. A total of 877 *Smicronyx* specimens were reared from infested plants during the study. *Smicronyx jungermanniae* (Reich) was the most abundant species, accounting for up to 96% of the total number of weevils reared from field dodder galls. *Smicronyx coecus* (Reich) and *Smicronyx smreczynskii* Solari were rare and accounted for only 5.6% and 1.0%. Larvae of *S. jungermanniae* and *Smicronyx smreczinskii* caused stem galls on *Cuscuta europaea* L. and *C. campestris* as well as seed destruction on *Cuscuta epithymum* (L.) L., and *C. europaea*. On the other hand, larvae of *S. coecus* were found in flowers and seeds of *C. epithymum* and *C. europaea* only. Infestation of field dodder by *Smicronyx* spp. ranged between 0% and 100%.

# Discussion

Larvae of *M. albocilia* exclusively mined the stems and root crowns of field bindweed in Slovakia. Spencer (1973) as well as Awadallah *et al*. (1976) stated the same. Rosenthal and Buckingham (1982) listed *C. arvensis* and also *Convolvulus althaeoides* as its hosts. *M. albocilia* therefore appears to be highly host specific on the target weed. Plants infested by *M. albocilia* looked healthy from the outside during its larval stage. The stems started to become weak and dry after pupation within the stems. In addition, exit holes may facilitate infection by diseases (Tóth, 2000). A complex

of seven hymenopterous parasitoids was shown to have a high impact on the populations of the stem-boring fly (Tóth *et al*., 2004b). Although infestation of field bindweed was high, parasitism reduced the agromyzid population by about 77%. In conclusion, *M. albocilia* could be an important biological control agent of field bindweed, especially in areas where the plant is invasive (e.g. North America), and parasitization of the fly would be expected to be lower.

Seven species of tortoise beetles were collected during the surveys in Slovakia. The predominant and only species attacking field bindweed was *H. subferruginea*. This species occurs on field bindweed throughout the Palearctic region (Kismali and Madanlar, 1990) and is unable to complete development on sweet potato (*I. batatas*; Rosenthal and Buckingham, 1982). In Slovakia, *H. subferruginea* was most frequent in warm and dry regions, less widespread in temperate regions and absent from cold regions (Tóth, 2000). In natural xerotherm ecosystems, the species often completely defoliated plants, while in cultivated crops, the beetles were not able to control their host plant. However, their effect was clear in vineyards with living green cover. Although predation and parasitization of tortoise beetles is mentioned as a major factor lowering their populations in the field, except for the egg parasitoid, *B. pungens*, no parasitoids of larvae and adults were recorded during our study. *H. subferruginea* was prioritized for the inundative biological control of field bindweed in Slovakia.

For common teasel, the tortricid *C. roseana* was selected as a potential classical biological control agent because of its ability to cause high seed reductions, its common occurrence in Slovakia and its distinct preference for *D. fullonum* (Cheesman, 1996). While Sforza (2002) concluded that *C. roseana* and *E. gentianaeana* should be similar in their potential as biological control agents for teasel, our results show that *E. gentianaeana* is not very promising because one larva damaged only about ten seeds.

Parasitic weeds (*Cuscuta* spp.) only reproduce by seeds. Thus, complete biological control of these weeds should be achievable by using organisms which damage the seeds. In the absence of species that kill the weeds at the seedling stage, the suppression of seed production is thus believed to be more important than damage to individual plants. Research efforts should also be directed to continue investigations on phytophagous arthropods, which can effectively be combined to provide maximum stress on parasitic weeds. *Smicronyx* spp. prevent flowering and fruiting of field dodder, either directly, through their feeding activity in the seed capsules, or indirectly, through weakening the shoots. If the stem of the species is not attached to its host plant beyond the attacked (galled) part, the entire section is killed (Baloch *et al*., 1967). Thus, *Smicronyx* is able to cause 100% seed reduction. In addition, they attacked field dodder over the whole growing season. We expect that *Smicronyx* species, above all *Smicronyx jungermaniae*, are very promising biological control agents of field dodder.

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# *Lewia chlamidosporiformans*, a mycoherbicide for control of *Euphorbia heterophylla*: isolate selection and mass production

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*Summary*

The potential of the fungus *Lewia chlamidosporiformans* Vieira and Barreto as a biological control agent for wild poinsettia, *Euphorbia heterophylla* L., a noxious invader of soybean fields in Brazil, is being assessed. One isolate was selected from nine that were tested as being the most aggressive to a series of wild poinsettia populations (including one known to be herbicide-resistant). The biphasic technique was investigated as an option for mass production of conidia of *L. chlamidosporiformans*. This method involves the production of mycelia in liquid culture that are later blended and poured into trays containing a solid medium and incubated under a specific light regime until conidia form. After 3 days, conidia are harvested once per day by pouring sterile water over the surface of the colonized medium and scraping the surface with a rubber spatula. A semi-synthetic liquid medium (with a sucrose and asparagin base) was selected as the best for the first phase of growth. A vegetable broth-agar medium supplemented with $CaCO_3$ was the best solid medium for fungal growth and sporulation in the second phase.

***Keywords***: bioherbicides, pathogenicity, biphasic.

## Introduction

Losses caused by weeds represent one of the main limiting factors in agriculture production worldwide. Chemical herbicide applications are gradually becoming the dominant method of control of weeds in both developed and developing countries (Wyse, 1992; Abernathy and Bridges 1994). However, parallel to this, problems with contamination of water resources, accumulation of chemical residues in the soil, emergence of herbicide resistence in weed species and threats to biodiversity are also on the rise. This justifies the search for alternatives that might allow the reduction or replacement of chemical herbicide applications such as through biological control of weeds with plant pathogens (Rosskopf *et al*., 1999).

Wild poinsettia (*Euphorbia heterophylla* L.), known in Brazil as 'amendoim-bravo' or 'leiteiro', is a native euphorb of tropical and subtropical America (Lorenzi, 2000). In Brazil, it is regarded as one of the worst weeds in important crops such as corn, sugarcane, common bean and soybean (Guedes and Wiles, 1976; Arevalo and Rozanski, 1991). The reduction in the soybean harvest caused by competition with *E. heterophylla* varies depending on the weed density in an invaded area and the soybean cultivar, but it is estimated that losses range from 35% to 62% (Constantin *et al*., 1997; Voll *et al*., 2002). Acetolactate synthase (ALS) inhibiting herbicides have been the favorite product used in the control of *E. heterophylla* in soybean. However, the repetitive use of these products and their residual effect in the soil led to a continuos selection of populations of *E. heterophylla* that are now resistant to these products (Gazziero *et al*.1998; Melhorança and Pereira, 1999). Such a situation offers an ideal opportunity for the use of a fungus formulated as a mycoherbicide (Charudattan, 2001).

*Lewia chlamidosporiformans* Vieira and Barreto is a newly described fungus capable of causing severe inflorescence necrosis, foliar blight and stem canker on *E. heterophylla* under natural conditions. Since its discovery, it has been intensively evaluated as a potential mycoherbicide. This paper reports some of the results obtained during these studies, namely isolate selection

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and development of a method for mass production of *L. chlamidosporiformans* inoculum.

# Materials and methods

## Fungal isolates

Samples of *E. heterophylla* with symptoms of attack by *Lewia* were collected in the Brazilian states of Minas Gerais, Rio de Janeiro, and Rio Grande do Sul.

Direct isolation from sporulating lesions as well as indirect isolations through surface sterilization and plating of diseased tissues was performed in vegetable-broth agar (VBA; Pereira *et al.*, 2003). Cultures were preserved in silica gel as described in Dhingra and Sinclair (1995).

## Inoculum production

Conidia of *L. chlamidosporiformans* of all fungal isolates were produced for the first isolate-selection experiment using the methodology described by Walker (1980) with the following modification: ten culture disks obtained from the margin of 7-day-old cultures grown in VBA were transferred to a series of Erlenmeyer flasks containing 100 ml of VB, i.e., the same as described in Pereira *et al.* (2003) but without agar. The erlenmeyers were left on a shaker at 140 rpm for 7 days at room temperature. After this period, the mycelial mass was blended within the remaining liquid medium within each flask and poured onto 20 × 28 cm aluminum trays, each containing 100 ml of solidified VBA. Trays were kept in a controlled temperature room at 26 ± 2°C under a 12-h photoperiod (light from two 40-W daylight fluorescent lamps and two 40-W fluorescent, near-ultra-violet light lamps). After 2 days, conidia were collected by pouring 50 ml of sterile water on the culture surface and scraping it with a rubber spatula. The resulting suspension was then filtered through two layers of cheese cloth, and the final concentration of the suspension was evaluated and adjusted to the adequate concentration for use in the experiment.

## *E. heterophylla* plants for the experiments

The populations represented in the experiment were produced from seeds obtained from different locations and included plants with the following characteristics: resistance to the herbicide imazetaphyr and resistance to *Bipolaris euphorbiae* (Hansford) Muchovej, a fungus previously evaluated as a mycoherbicide for wild poinsettia (Yorinori and Gazziero, 1989; Marchiori *et al.*, 2001, Nechet *et al.*, 2006). Seeds to be used in the experiments (Table 1) were harvested from healthy plants grown in a greenhouse and stored at 5°C until use. Plants used in the experiments were produced from pre-germinated seeds that were planted in 500-ml pots containing sterile soil. The plants were maintained in a greenhouse (26 ± 2°C) and watered daily. Plants were inoculated at the three- to four-leaf stage.

## Screening of fungal isolates

Groups of plants of nine populations listed in Table 1 were inoculated with conidial suspensions representing each isolate obtained in the survey. Inoculum consisted of suspensions of $1.0 \times 10^4$ conidia/ml + 0.05% Tween 20 (polyoxyethylene monolauratic) + 0.05% Breakthru® (polyether-polymethyl siloxane copolymer + polyether; T.H. Goldschmidt, Guarulhos, São Paulo). After inoculation, plants were kept in a mist room at 25°C for 24 h and then moved to a greenhouse (26 ± 2°C). Plants inoculated with a suspension with the same components as described above but without *L. chlamidosporiformans* conidia served as the control. The number of dead plants and percentage of diseased leaves (proportion of number of diseased leaves per total number of leaves) were evaluated at 5-day intervals for 30 days, and the area under the disease progress curve (AUDPC) was estimated (Campbell and Madden, 1990).

The experiment was carried out in a completely randomized design with a factorial of nine isolates, nine plant populations and three replications per treatment. Each replicate consisted of one pot containing two plants.

**Table 1.** *Euphorbia heterophylla* populations included in the study.

| Code | Origin |
|---|---|
| EKLN16 | Niterói-RJ |
| EKLN19 | Viçosa-MG |
| EKLN247 | Itabuna-BA |
| ERWB274 | Nova Laranjeira-PR |
| ERWB280 | Nova Petrópolis-RS |
| ETSB | Londrina-PR |
| ETRB | Londrina (resistant to *B. euphorbiae*) |
| ESH | Viçosa-MG |
| ERH | Viçosa-MG |

## Evaluation of liquid-culture media on the mycelial growth of *L. chlamidosporiformans*

This experiment aimed to evaluate the growth of *L. chlamidosporiformans* (isolate KLN-06) in a series of five common liquid-culture media of different compositions (see below for details) at either standard or double concentration. This test aimed to determine the composition of a liquid medium, from among the following, that might be adequate for mass production of mycelium for the first stage of a biphasic-technique (Walker, 1980):

1. PS (Dhingra and Sinclair 1995, standard concentration): decoction of 200 g potato; 20 g sucrose; 1 l distilled water;
2. PS × 2 (doubled concentration): decoction of 400 g potato; 40 g sucrose; 1 l distilled water;
3. VBS (VB as mentioned above supplemented with sucrose)-100 ml vegetable broth; 20 g sucrose; 450 ml distilled water;
4. VBS × 2 (doubled concentration)- 200 ml vegetable broth; 40 g sucrose; 450 ml distilled water;
5. Marine ammonium mineral salt (MAMS; standard concentration): decoction of 200 g castor-bean plant leaves; 20 g sucrose; 1 l distilled water;
6. MAMS × 2 (doubled concentration): decoction of 400 g castor-bean plant leaves; 40 g sucrose; 1 l distilled water;
7. MANDS (standard concentration): decoction of 200 g cassava leaves; 20 g sucrose; 1 l distilled water;
8. MANDS × 2 (doubled concentration): decoction of 400 g cassava leaves; 40 g sucrose; 1 l distilled water;
9. MSSA: semi-synthetic sucrose-asparagin medium (Alfenas, 1998; normal concentration): 10 g sucrose, 2 g L-asparagin, 2 g yeast extract; 1 g $KH_2PO_4$ ; 0.1 g $MgSO_4{\cdot}7H_2O$; 0.44 mg $ZnSO_4{\cdot}7H_2O$; 0.48 mg $FeCl_3{\cdot}6H_2O$; 0.36 mg $MnCl_2{\cdot}H_2O$; 1 l distilled water.
10. MSSA · 2 (doubled concentration): 20 g sucrose, 4 g L-asparagin, 4 g yeast extract; 2 g $KH_2PO_4$; 0.2g $MgSO_4{\cdot}7H_2O$; 0.88 mg $ZnSO_4{\cdot}7H_2O$; 0.96 mg $FeCl_3{\cdot}6H_2O$; 0.72 mg $MnCl_2{\cdot}H_2O$; 1 l distilled water.

Three mycelial plugs from 7-day-old cultures grown on PDA were transferred to 125-ml Erlenmeyer flasks, each containing 50 ml of one of the liquid-culture media described above. The flasks with plugs were placed on a shaker at 100 rpm at room temperature (25°C). Dry weight of mycelia produced was evaluated after 7 days of incubation. The contents from each flask were vacuum filtered through filter paper until the mycelium was dry. The mycelial mass was then scraped and weighed after drying in an electric oven at 70°C for 24 h. The experiment had a completely randomized design with four replications.

## Effect of different solid-culture media on sporulation of *L. chlamidosporiformans*

This experiment aimed to determine the influence three different solid-culture media (supplemented or not with $CaCO_3$ at 3 g/l), on the sporulation of *L. chlamidosporiformans* during the second growth phase of the biphasic system of mass production. The following culture media were tested:

1. Concentrated PCA: decoction of 200 g potato; 200 g carrot; 1 l distilled water; 20 g agar;
2. Concentrated PCA + $CaCO_3$
3. VBA (Pereira *et al.* 2003)
4. VBA + $CaCO_3$
5. FLA: decoction of 200 g 'wild poinsettia leaves', 20 g sucrose, 1 l distilled water.
6. FLA + $CaCO_3$

The medium utilized during the first phase (mass production of mycelium in liquid culture) was MSSA (described above), and the procedure was also as described above. The mycelium was blended inside of the erlenmeyers, and 100 ml of the resulting suspension was poured into each of 24 aluminum trays (35 × 20 cm), containing 100 ml of the solid media that were being tested. Trays were kept in a controlled temperature room at 26 ± 2°C under a 12-h photoperiod. After 3 days, conidia were harvested once per day by pouring 50 ml of sterile water over the surface of the colonized medium and scraping the surface with a rubber spatula, with a total of four harvests per tray. The obtained suspension was filtered through three layers of cheesecloth. An aliquot of 20 μL of the conidial suspension obtained from each tray was removed and mounted on a microscope slide, and the number of conidia produced was counted, with results converted into conidia/ml from each treatment. The experiment had a completely randomized design with four replications and each tray represented a replicate. Statistical analysis was made of the sum of conidial concentrations obtained for the four harvests.

# Results

## Selection of a fungal isolate

Nine isolates were obtained from several locations (Table 2). Among these isolates, only three were pathogenic to all *E. heterophylla* accessions that were screened. Isolate KLN06 caused the highest disease-intensity levels resulting in larger values of AUDPC for five of the wild poinsettia populations involved in the test, including population ERH (resistant to the herbicide imazethaphyr) and was equivalent to other isolates in disease severity caused to the remaining weed

**Table 2.** *Lewia chlamidosporiformans* isolates included in the study.

| Code | Origin |
|---|---|
| KLN06 | Viçosa-MG |
| KLN09 | Araruama-RJ |
| KLN14 | Italva-RJ |
| KLN15 | Niterói-RJ |
| KLN17 | São Miguel do Anta-MG |
| KLN18 | Viçosa-MG |
| KLN19 | Viçosa-MG |
| KLN20 | Viçosa-MG |
| RWB280 | Nova Petrópolis-RS |

populations (Figure 1). The first symptoms appeared 5 days after inoculation with KLN06, and plant death started appearing 7 days after inoculation in populations EKLN19, ERWB247, ETSB, ERH and ESH. KLN06 was selected as the most promising isolate for further studies.

## Evaluation of liquid-culture media on the mycelial growth of *L. chlamidosporiformans*

The liquid-culture media that resulted in the hightest levels of *L. chlamidosporiformans* mycelial production was MSSA × 2 (Figure 2). Other media yielded inferior results for the production of *L. chlamidosporiformans* mycelia, reaching values that varied from 1/3 to 1/2 that obtained with MSSA × 2. A smaller production of mycelial mass was obtained for: PS, MAMS and MAMS × 2. The doubled concentration of the ingredients in the liquid-culture media only resulted in significant increase in the yield of mycelial biomass for MSSA × 2 and PS. Mycelial production for MSSA × 2 was triple that obtained for standard MSSA (Figure 2). MSSA × 2 was hence selected for use as medium in the first phase of biphasic mass production of *L. chlamidosporiformans*.

## Effect of different solid-culture media on sporulation of *L. chlamidosporiformans*

Results obtained in this experiment are presented in Figure 3. Among the solid-culture media that were tested for sporulation in the second phase of the biphasic mass production of *L. chlamidosporiformans*, VBA + $CaCO_3$ had the best performance. Its use resulted in a production of $8.1 \times 10^5$ conidia/ml and was followed by PCA + $CaCO_3$ ($7.03 \times 10^5$ conidia/ml) and FLA + $CaCO_3$ ($5,6 \times 10^5$ conidia/ml). There were significant statistical differences among most culture media being tested (Figure 3). The supplementation of $CaCO_3$ (3g/l) significantly increased the production of spores of the fungus for all solid-culture media being tested, and this

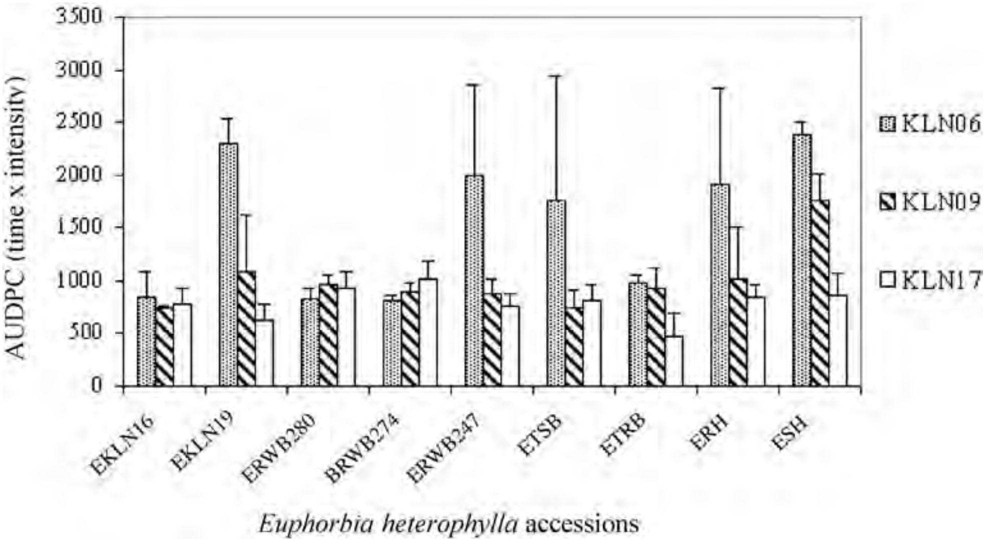


**Figure 1.** Area under the disease progress curve for the isolates KLN06, KLN09, and KLN17 of *Lewia chlamidosporiformans* based on disease severity (means of three repetitions; *bar* standard deviation).

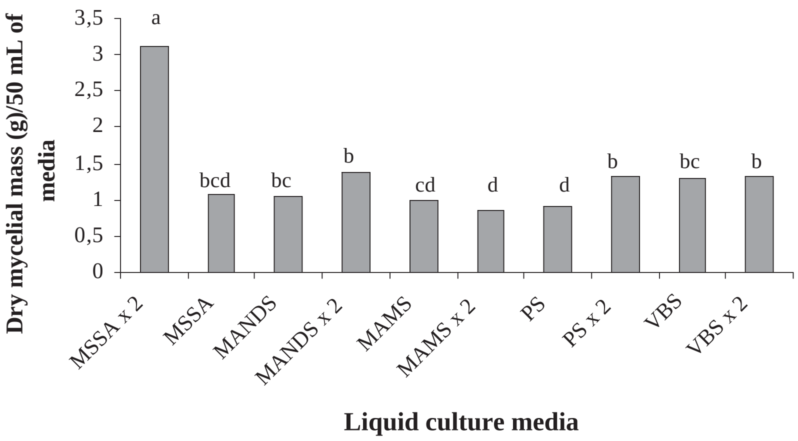


**Figure 2.** Production of mycelial mass of *Lewia chlamidosporiformans* in different liquid-culture media (means of four repetitions, *bars* standard deviation, *means followed by the same letter* did not differ under Tukey test at the level of 5% of probability).

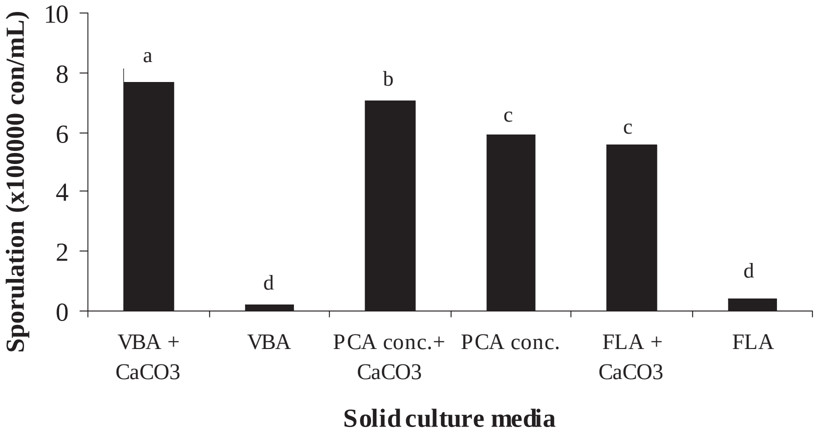


**Figure 3.** *Lewia chlamidosporiformans* conidial production on different solid-culture media (means of four repetitions, *bars* standard deviation, *means followed by the same letter* did not differ under Tukey test at the level of 5% of probability).

was particularly significant for VBA. The addition of $CaCO_3$ (3g/l) increased conidial production in VBA 16-fold. It also increased conidial production for FLA by a factor of 9.3 and by a factor of 1.24 for PCA.

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# *Sphenoptera foveola* (Buprestidae) as a potential agent for biological control of skeletonweed, *Chondrilla juncea*

**M.G. Volkovitsh,[1] M.Yu Dolgovskaya,[1] S.Ya Reznik,[1] G.P. Markin,[2] M. Cristofaro[3] and C. Tronci[4]**

*Summary*

Skeletonweed, *Chondrilla juncea* L. is an important invasive weed in the USA, Australia, and Argentina. With the aim of finding new potential agents for biological control of this weed, surveys were carried out in 2004 to 2005 in its native range in Southern Russia and Kazakhstan, where the bronze skeleton weed root borer, *Sphenoptera foveola* (Gebler) (Coleoptera: Buprestidae) was repeatedly collected from different *Chondrilla* species. According to the literature and our survey, this buprestid is widely distributed in sandy deserts of Southern Russia and Kazakhstan. Locally, it could be rather abundant. Observations suggest that both larvae and adults of *S. foveola* feed exclusively on plants of the genus *Chondrilla.* Adults feed on green stems, larvae feed externally (within latex case) on roots and, at high population density, can cause significant damage to attacked plants. We conclude that *S. foveola* should be considered as a potential agent for biological control of skeleton weed, although further studies (particularly, host-specificity tests) are necessary to prove this hypothesis. *Sphenoptera (Deudora) clarescens* Kerremans, another sphenopteran species attacking *Chondrilla* in Iran and Turkey, may have a different root-feeding strategy and invites further investigations to evaluate it as a potential agent for biological control of skeleton weed.

***Keywords***: bronze skeleton weed root borer, surveys, taxonomy, distribution, biology, host range, impact.

## Introduction

*Chondrilla juncea* L. (Asteraceae), skeletonweed, is an important invasive weed in the western USA, Australia, and Argentina. With the aim of finding potential agents for biological control of this weed, extensive surveys have been carried out in 2004 and 2005 in its native range in Southern Russia and Kazakhstan. Among other phytophagous insects, the bronze skeleton weed root borer, *Sphenoptera foveola* (Gebler, 1825) (Coleoptera: Buprestidae) was repeatedly collected from different *Chondrilla* species. In an earlier programme this buprestid had been considered as a potential candidate for biological control (Caresche, 1970; Wapshere, 1973, 1974). However, no attempts were made to test this species. Another root boring buprestid, *Sphenoptera (Deudora) clarescens* Kerremans, 1909 distributed in Iran and Turkey was considered and tested, but dropped as a potential biological control candidate (Hasan, 1978). From a biological aspect *S. foveola* is one of the best studied species of *Sphenoptera* due to the ability of its larvae to induce latex secretion from *Chondrilla* roots. In the 1930s, an ambitious project was conducted in the former Soviet Union to use native Central Asian latex-producing plants (mainly *Scorzonera, Chondrilla,* and *Taraxacum*) for rubber production. This project resulted in extensive investigations of selected plants and associated insects including *S. foveola* (Emelianova *et al*., 1932).

To evaluate the potential perspectives of *S. foveola* as a candidate for biological control of *C. juncea*, field collections and biological observations were made in 2004 to 2005 in Kazakhstan and Russia.

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## Methods and materials

The first trip to Kazakhstan was made between June 23 and July 15, 2004 and included two 6-day field trips and several 1-day visits. Total extent of the route covered was 4230 km, in which 41 sites were surveyed and *S. foveola* was found at eight. The second trip from May 10 to May 22, 2005 included one 7-day field trip and two short daily visits; total distance covered was approximately 1500 km with 18 sites being visited at which *S. foveola* was found at four. These trips covered mainly low land and foothill desert areas (elevation, 400–1385 m) between Ili River on the west, the artificial Qapshagay Lake on the south and Balqash Lake on the north (Figure 1). Numerous species of *Chondrilla* were abundant in the study area. Adults of *S. foveola* were collected by hand from *Chondrilla* plants or from the soil under the plants where they were apparently ovipositing. To collect preimaginal stages, the roots of different *Chondrilla* species were excavated, examined and if necessary, dissected.

To study the geographic distribution of *S. foveola,* the buprestid collection of Laboratory of Insect Taxonomy, Zoological Institute, Russian Academy of Sciences, St. Petersbourg, Russia (ZIN) was examined, all collecting data were studied and collecting localities mapped (Figure 1). Confirmation of identification was performed by comparison with syntype from ZIN collection using a dissecting microscope. *Chondrilla* species collected were identified by botanists from Botanical Institute, Russian Academy of Sciences, St. Petersbourg, Russia, and A. Popov, Volgograd, Russia.

## Results and discussion

### Taxonomy

Taxonomical position of this buprestid is as follows: Coleoptera: Buprestidae: Chrysochroinae: Sphenopterini (Volkovitsh and Kalashian, 2006).

*Sphenoptera (Sphenoptera) foveola* Gebler, 1825: 46 (as *Buprestis*). 1 Syntype: Steppe Kirgiz., Gebler (Zoological Museum, Helsinki University, Finland); 1 syntype: the same label (ZIN).

Synonym: *strandi* Obenberger, 1920: 113. 2 syntypes: Tarbagatai, Siberia [Tarbagatai Mts., Kazakhstan] (National Museum, Praha, Czech Republic).

Synonym: *foveola* var. *usta* Obenberger, 1927: 51. 1 syntype (female): Astrakhan (National Museum, Praha, Czech Republic).

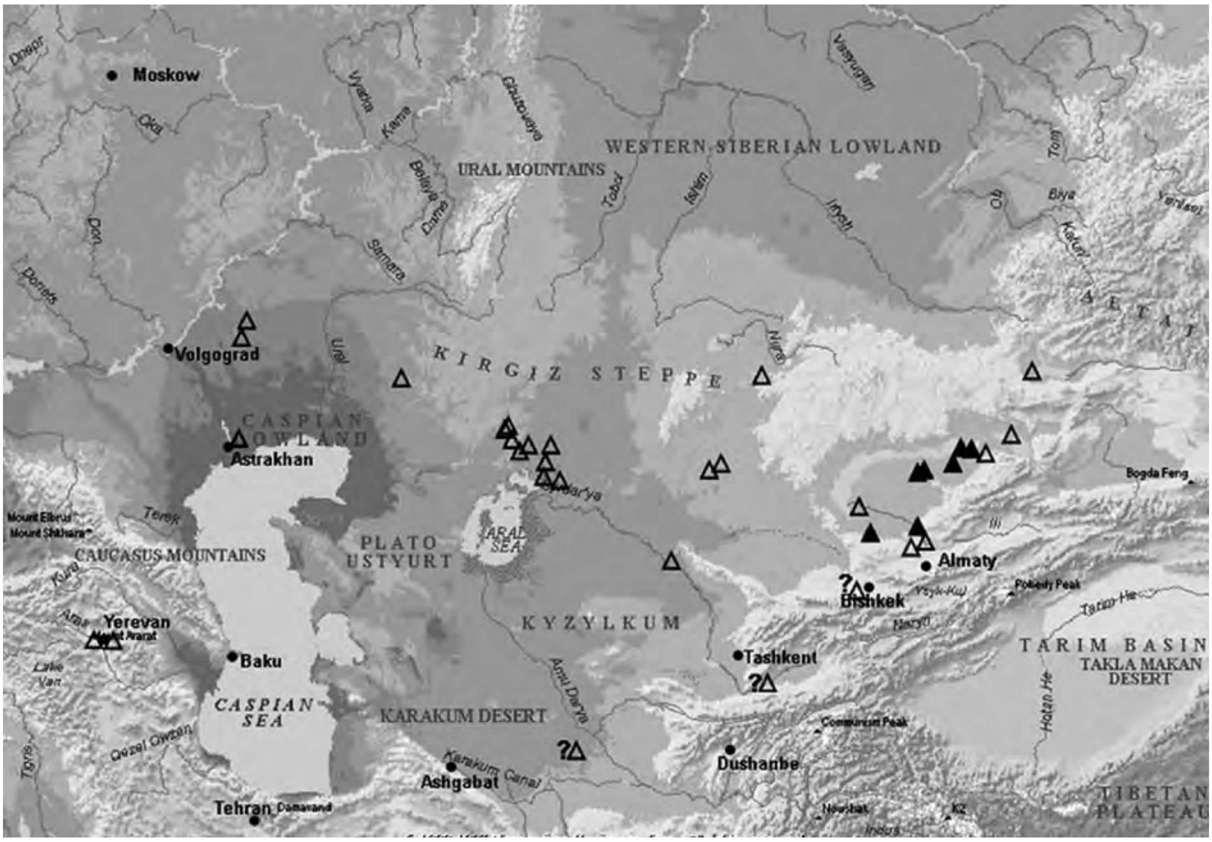


**Figure 1.** The distributional map of bronze skeleton weed root borer, *Sphenoptera foveola*. *Closed triangles* refer to our field collections in 2004 to 2005; *open triangles* refer to the data from the insect collection of Zoological Institute, St.Petersburg, Russia.

Larva: Alexeev *et al*., 1990 (detailed morphological description).

We looked for but did not find another sphenopteran species, *S*. (*Deudora*) *clarescens* Kerr., reported by Hasan (1978), to be found in Iran and Turkey by Kashefi (2002) and C. Tronci (unpublished communication). This species tunnels inside the roots of *C. juncea* which is different from that of *S. foveola* which feed externally on the roots. *S. clarescens,* according to Hasan (1978), also has a very wide host range among non-*Chondrilla* genera of plants which would make it an unsuitable biological control agent. We did not find evidence of a *Sphenoptera* that fit the description of *S. clarescens* in our surveys in either Kazakhstan or southern Russia or find evidence in museum collection of it having been collected in any former USSR countries.

## Geographical distribution

According to the literature, *S. foveola* has been found in southeastern European Russia (Astrakhan prov.), Armenia, Kazakhstan, Kyrgyzstan, Uzbekistan, Turkmenistan (Alexeev *et al*. 1990, Kadyrbekov and Tleppaeva 2004, Volkovitsh and Kalashian 2006). The great majority (28 specimens examined) were from east of the Caspian Sea or the Volga River (Figure 1). We did not find any specimens from the coastal sandy semi-desert areas around the west side of the Caspian Sea but we suspect that it would be found in this area as well as in the semi-desert and desert habitats along the valleys of the Kura and Arax rivers in the Armenian Mountains. This is based upon the finding of two specimens of *S. foveola* in the ZIN collection from Armenia, the only two specimens from west of the Caspian Sea.

## Biology

Our field observations and the literature (Emelianova *et al*., 1932) suggested that adult *S. foveola* are present in the field from mid-April to mid-October, where they feed on growing *Chondrilla* plants in hilly, sandy deserts (Figures 2, 3a). In the hottest part of the day we found adult beetles usually sitting, head down on the stems of *Chondrilla* plants or in the plant's shadow on the ground probably to protect themselves from overheating. Under laboratory conditions, oviposition was observed from mid-May until the beginning of October. In the field, eggs were laid in the sand near the crown or directly on the crown of the plant. In the laboratory, oviposition of an individual female lasted 10 to 50 days during which she laid 11 to 135 eggs. In the field, peak oviposition activity appeared to occur in June and July. The eggs take 12 to 14 days to develop in July but more than 30 days in September. Mortality of eggs laid both in the laboratory and collected in the field was approximately 25%. By the end of the summer, 70% of the plants examined had been attacked but in some sites this could reach 90% to 97% ($n > 50$).

Newly eclosed, first instar larvae migrate down the outside of the taproot working their way down between the grains of sand to a depth of two to three centimeters where they chew into the cortex on the *Chondrilla* roots. Their attack provokes an extreme secretion of latex which when mixed with sand forms a small, porous case one to one and one-half cm long in which the larvae develop. As the larvae grow, they move spirally down the outside of the root, further damaging the cortex and releasing more latex which increases the size of the case, which can now surround the root (Figures 3b, c). A single case can be up to 30 cm long and often when several larvae attack the same plant and their combined cases fusing forming congealed latex/sand lump that can be as big as 225 grams. It was noticed that plants growing in loose stands have both a higher rate of attack and larger than in plants growing in compact, hardened sand. The cases contain hollow chambers in which the larvae move freely and extend from near the surface to over 30 cm deep. At the deepest points, they probably experience a constant temperature of 20°C to 25°C which may be optimal for larval development. In the field, larvae usually eclose in May or June, feed during the summer, over-winter as larvae inside the case, and pupate the following spring. Adults emerge from the pupae at the beginning of summer, usually June and July. Larvae that hatch from eggs laid in midsummer over-winter and continue to feed in the following season. However, larvae from the first eggs laid in spring may complete their development and produce adults by the end of the summer which then may over-winter.

## Host range

According to our field observations,*S. foveola* fed and developed mainly on *Chondrilla ambigua* Fisch. ex Kar. et Kir., and, occasionally, on *C. canescens* Kar.

**Figure 2.** Typical collection site for the bronze skeleton weed root borer, *Sphenoptera foveola,* in hilly desert. Kazakhstan, Site KZ04-08, Almaty Region, Qumbasy sandy desert, 69 km NNW of Qapshagay, 28.06.2004.

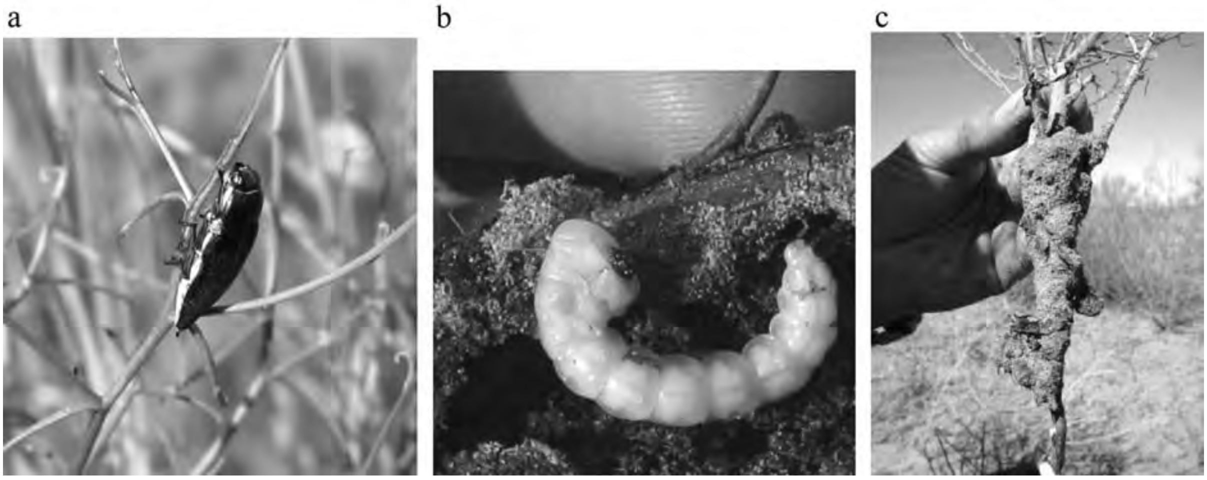


**Figure 3.** The bronze skeleton weed root borer, *Sphenoptera foveola*: (a) the adult; (b) mature larva; and (c) latex and sand cases of the mature larvae. The insect was feeding on *Chondrilla ambigua*, Almaty Region, Kazakhstan.

et Kir. Additionally, *S. foveola* was reported from the literature to have been found feeding on *C. pauciflora* Ldb. and more rarely on *C. brevirostris* Fisch. et Mey. (singularly) (Emelianova *et al*., 1932), but not on *C. juncea*. The genus *Chondrilla*, is taxonomically divided into several divisions, section *Brachyrhynchus* contains *C. ambigua* and *C. pauciflora.* Section *Euchondrilla* contains *C. brevirostris* and *C. canescens* which also contains *C. juncea* L. (Iljin, 1930, Flora SSSR, 1964).

*S. foveola* feeding on a non-*Chondrilla* species, *Scorzonera tau-saghyz* Lipschiz et Bosse, was reported by Alexeev *et al*. (1990) although they give no data to support this claim. This doubtful host use was repeated by Tleppaeva (1999), Kadyrbekov and Tleppaeva (2004), and Tleppaeva and Ishkov (2004) but without any additional substantiating data. Our field observations agree with Emelianova *et al*. (1932) who suggested that only *Chondrilla* species are suitable for *S. foveola* larval development, with feeding primarily concentrated on species of the *Brachyrhynchus* section with fewer records from *Chondrilla* in the *Euchondrilla* section.

## Impact on the host plant

Chewing by adults on *Chondrilla* shoots often kills small branches which break off when dry. However, most of the damage is done by larvae feeding on the roots. The damage from a single small larva is usually not fatal but damage by mid-sized larva particularly if there are several, often kills the above ground portion of smaller plants and some times larger stems of bigger plants. The copious flow of latex exuded by the wounded roots represents a major loss of nutrients and energy. This loss of latex if does not kill the plant, must stress it, which should result in reduced growth, flowering, and general loss of competitiveness. Also, increased stress from the loss of latex probably would weaken the plants making them more susceptible to the three existing biological control agents already established in many parts of *Chondrilla juncea*'s introduced range (Julien and Griffiths, 1998).

# Discussion

*S. foveola*'s natural range, at least where it is most abundant, seems to extend from the Volga River and Caspian Sea eastward through the deserts of Kazakhstan, possibly northern most parts of Turkmenistan and Uzbekistan, as well as the southern most part of Russia adjacent to Kazakhstan. This area is probably the centre of origin for the genus *Chondrilla* since it contains approximately 18 of the 21 known species of this plant (Flora SSSR, 1964). By contrast, the target weed, *C. juncea* is one of the few species not found in this area. *C. juncea*'s range extends eastward across Europe from Spain, along the borders of the Mediterranean Sea (Wapshere *et al*., 1974). It is also found in the Balkans, Turkey and Iran and along the north shore of the Black Sea (Wapshere *et al*., 1976). The recorded eastern edge of its range ends approximately at the edge of the Caspian Sea and the Volga River (our review of herbarium species). It is therefore unfortunate that the range of *S. foveola* does not naturally extend westward far enough for it to overlap that of *C. juncea*.

The wide range of other species of *Chondrilla* which *S. foveola* can attack including the very closely related species *C. brevirostris* and *C. canescens* make us suspect that under the right climate conditions, *S. foveola* will probably also attack *C. juncea*. We are presently planning feeding studies to determine the suitability of *C. juncea* as a new association host for *S. foveola*. The fact that *S. foveola* can attack a number of different species of *Chondrilla* should not prohibit it from being considered as a biological control agent. In all parts of the introduced range of *C. juncea* there are no native or introduced species of *Chondrilla* which might be

at risk from this attack. Its potential for attacking species other than in the genus *Chondrilla*, we feel is also low since we found the only record in the literature being *Scorzonera tau-saghyz* reported by Alexeev *et al*., (1990). We hope to address the question of its potential for non-*Chondrilla* host attack in future host testing. We are also planning to find and compare *S. foveola* with the related species of *Sphenoptera clarescens* reported attacking *Chondrilla* in Turkey and Iran.

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# Common buckthorn, *Rhamnus cathartica* L.: available feeding niches and the importance of controlling this invasive woody perennial in North America

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*Summary*

Common buckthorn, *Rhamnus cathartica* L., an invasive woody perennial of northern hardwood forests in North America, has been targeted for classical biological control, and research has been underway since 2001. In support of biological control research, a survey was conducted for insects associated with common buckthorn in a portion of its introduced range in the state of Minnesota. This survey provides baseline information on available feeding niches for potential control agents of common buckthorn and identifies the natural enemy community that could potentially interfere with agent establishment. In 2 years of sampling, 356 species representing 111 families and 13 orders were collected from common buckthorn in Minnesota. There was no significant defoliation observed at any of the study sites. We surmise that ample feeding niches are available given that most herbivores collected can be classified as generalists. However, the abundance of parasitoids and predators may hinder establishment of potential biological control agents. Further research is needed to determine if biotic resistance could play a significant role in preventing establishment of herbivores in a classical biological control programme for common buckthorn in North America.

***Keywords***: Rhamnaceae, arthropod herbivores, natural enemies, biological control.

## Introduction

Common buckthorn, *Rhamnus cathartica* L., is an invasive woody perennial that has become established in northern hardwood forests of North America. It was introduced as a landscape plant and used as a shelterbelt tree because of its winter hardiness and its ability to grow in multiple soil types and habitats (Archibold *et al*., 1997). In North America, common buckthorn is one of the most invasive woody perennials in natural ecosystems (Archibold *et al*., 1997; Catling, 1997). Common buckthorn retains its leaves longer than native tree species, creating a competitive advantage (Harrington *et al*., 1989). In addition, Archibold *et al*. (1997) suggested that common buckthorn might be allelopathic, allowing its seedlings to grow below mature female trees while inhibiting native tree species. Common buckthorn produces a dense branching structure that attracts nesting songbirds; however, the American robin, *Turdus migratorius* L., experiences higher levels of predation when nesting in common buckthorn compared to native species (Schmidt and Whelan, 1999). Others have documented that common buckthorn causes changes in soil properties, leaf litter composition, and micro-arthropod communities (Heneghan *et al*., 2002, 2004).

Common buckthorn has negative impacts on agriculture. It is the spring host for oat crown rust, *Puccinia coronata* Corda, which can cause severe yield losses in oats (Harder and Chong, 1983). Common buckthorn was identified as a suitable overwintering host for soybean aphid, *Aphis glycines* Matsumura, which was first discovered in North America in 2000 and by 2007 has spread to 24 states and three Canadian provinces (Ragsdale *et al*., 2004; Voegtlin *et al*., 2005).

Common buckthorn is currently on the noxious weed list in six states and two Canadian provinces (University

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of Montana-Missoula, 2007; USDA, 2007). Multiple methods of control have been employed against common buckthorn including cut stump treatments, foliar herbicide applications, and burning (Archibold *et al*., 1997). Such control efforts are expensive and for the most part are only effective on a small scale because seedlings tend to re-grow after a burn or a chemical treatment (Archibold *et al*., 1997). In the early 1960s, several potential biological control agents were identified by Malicky *et al*. (1970), but their study was not continued. In 2001, biological control research was resumed by the Minnesota Department of Natural Resources in collaboration with CABI Europe Switzerland to identify and screen potential control agents.

Our first objective was therefore to conduct a survey of herbivorous insect species associated with common buckthorn, while our second objective was to identify which predators and parasitoids could be found on common buckthorn in Minnesota. These data will provide key information in understanding the availability of feeding niches for potential biological control agents and provide insights on what biotic resistance might be present to interfere with agent establishment in Minnesota.

# Methods and materials

## Field sites

In 2004 and 2005, eight common buckthorn sites were sampled in three different habitat types, i.e., urban (three sites), rural (two sites), and agricultural (three sites), in seven (2004) or six (2005) southern Minnesota counties (see Yoder 2007 for specific locations). Sites were characterized for their plant communities by randomly sampling ten 1-$m^2$ plots. Data collected in each plot included: percent cover of common buckthorn, percent cover of other plant species, common buckthorn stem density, other plant species stem density, number of different plant species, and percent canopy cover. Canopy cover was estimated using a densiometer. To characterize mature trees in the forest, which were not captured by the 1-$m^2$ plots, we counted the number of trees for each species that were at least 1.5 m in height, in a 2-m radius around each 1-$m^2$ plot.

## Insect sampling

In 2004 and 2005, 12 common buckthorn plants: four small (<1m in height), four medium (1–3 m), and four large (>3 m), were marked for repeated insect sampling at each site. Sites were visited every 2 weeks throughout the growing season (15 June–15 September 2004; 15 May–15 September in 2005). All reachable branches were visually surveyed and any insect present was collected, and immediately returned to the laboratory for either identification if adults were captured or reared to adult stage if immature insects were collected. In addition to the 12 plants sampled biweekly, two transects were established at either five (2004) or six (2005) of the largest sites. The first transect consisted of 25 consecutive common buckthorn trees growing along a path, roadway, or other opening where trees had full exposure to the sun resulting in common buckthorn trees that were larger. The second transect was perpendicular to the first transect and consisted of another 25 consecutive common buckthorn trees and included trees growing in the under-story in shade or filtered sunlight. All trees selected were visually surveyed for up to 2 min and all insects observed were collected and returned to the laboratory.

Adults reared and collected in the field were preserved and pinned for later identification. Soft-bodied insects and immature insects that failed to reach the adult stage were preserved in vials containing 70% ethanol. Voucher specimens were deposited in the Entomology Museum at the University of Minnesota.

All adult specimens were categorized as herbivores, predators, parasitoids, or scavengers. For a species to be included in the statistical analysis, a minimum of five specimens per species was required. A qualitative Sorenson index (Magurran, 1988) was used to characterize differences in insect assemblages between habitat types. The equation for the qualitative Sorenson index ($C_S$) is $C_S = 2j/(a + b)$ where j is the number of species found in both groups, a is the number of species in group x, and b is the number of species in group y. We used a quantitative Sorenson index (Magurran, 1988) to characterize differences in insect assemblages in relation to abiotic factors such as the amount of sunlight (forest edge vs. interior) and biotic factors such as tree size (small, medium, large). The equation for the quantitative Sorenson index ($C_N$) is $C_N = 2_{jN}/(aN + bN)$ where *jN* is the sum of the lower of the two abundances recorded for a given species found in both groups, aN is the total number of specimens in group *x*, and *bN* is the total number of specimens in group *y*. The closer $C_S$ or $C_N$ are to 1, the more similar the groups are, and the closer to 0, the more dissimilar.

# Results

## Site characteristics

Overall, urban sites had the highest density of common buckthorn and the lowest plant species diversity (Table 1). Those sites characterized as agricultural sites had the opposite, with the lowest density of common buckthorn and the greatest plant diversity (Table 1). Those sites characterized as rural had an intermediate percent cover of common buckthorn, but on a stem density per square metre had common buckthorn densities equal to those of the urban landscapes. Plant species diversity was low in the rural sites, but the percent cover of other plant species and stem density of other plant species was intermediate (Table 1). Interestingly,

**Table 1.** Site characteristics for three habitat types (urban, rural, and agricultural) surveyed for insect fauna on *Rhamnus cathartica,* common buckthorn.

| Site characteristics | Urban sites | Rural sites | Agricultural sites |
|---|---|---|---|
| All vegetation (1 m$^2$) | | | |
| % Cover of common buckthorn | 61.0 ± 0.1 | 48.0 ± 0.1 | 31.0 ± 0.1 |
| % Cover of other plant species | 39.0 ± 0.1 | 52.0 ± 0.1 | 69.0 ± 0.1 |
| Common buckthorn stem density m$^2$ | 6.1 ± 1.1 | 6.1 ± 1.1 | 4.3 ± 0.9 |
| Other plant species stem density m$^2$ | 11.5 ± 2.4 | 28.4 ± 3.4 | 26.4 ± 3.6 |
| Number of other plant species | 3.5 ± 0.4 | 3.8 ± 0.3 | 6.8 ± 0.5 |
| % Canopy cover | 82.0 ± 1.1 | 83.0 ± 0.8 | 80.0 ± 0.8 |
| Mature tree survey (12.56 m$^2$)[a] | | | |
| Number of common buckthorn trees | 6.1 ± 1.5 | 2.8 ± 0.7 | 1.1 ± 0.3 |
| Number of other trees | 1.9 ± 0.4 | 1.4 ± 0.2 | 2.6 ± 0.3 |
| Number of other tree species | 1.5 ± 0.3 | 1.1 ± 0.1 | 1.4 ± 0.1 |

[a] Trees at least 1.5 m tall in a 2-m radius from center of plot (12.56 m$^2$).

if common buckthorn was excluded from this analysis, the most common plant species found in either urban or rural sites was garlic mustard, *Alliaria petriolata* L., another invasive plant of hardwood forests. When surveying the mature tree composition, four sites had common buckthorn as the dominant mature tree. Urban sites had a significantly higher density of mature common buckthorn trees when compared to rural or agricultural sites with one urban location having a maximum of 11 mature buckthorn trees per 1 m$^2$. Agricultural sites had the lowest number of mature buckthorn trees and the highest number of other mature tree species (Table 1). For all sites, the most dominate mature tree species, other than common buckthorn, was American elm, *Ulmus americana* L., followed by box elder, *Acer negundo* L.

## Insect fauna

Over the 2-year study, a total of 1733 arthropods representing 13 orders, 111 families and 356 species were collected from common buckthorn. Hemiptera was the most abundant order, followed by Hymenoptera, which consisted mostly of parasitoids (Tables 2 and 3). Several species were abundant, each with over 75 specimens collected: *Metcalfa pruinosa* (Say) (Flatidae), *Lasius alienus* (Förster) (Formicidae), *Harmonia axyridis* (Pallas) (Coccinellidae), *Graphocephala coccinea* (Forster) (Cicadellidae), and *Trissolcus* sp. a. (Scelionidae).

For the analysis we used 606 herbivores representing 32 different species, 154 predators representing five different species, and 140 parasitoids representing four different species (Tables 2 and 3). An additional 314 species were excluded from analysis because fewer than five specimens were collected over the 2-year sampling effort or because species were known to be saprophagous, mycetophagous, scavengers, or non-feeding as adults. The Sorenson index ($C_S$) showed that all three habitat types, agriculture, rural, and urban landscapes, were very similar in insect species diversity (range 0.71–0.73). The majority of predators were captured at sites in agriculture habitats (56%) and the majority of parasitoids were captured in rural habitats (61%). The quantitative Sorenson index for insect diversity for forest edge and interior using data collected from the two perpendicular transects was ($C_N$ = 0.54). It is not surprising that transects are different since more insects were collected on common buckthorn along the transect where plants were along a forest edge (62% of captures) compared to the interior (38% of captures). When comparing tree sizes, large and small trees had the least similar insect composition ($C_N$ = 0.44); whereas medium and small trees had the most similar insect composition ($C_N$ = 0.59). Medium trees tended to have higher diversity and abundance compared to large and small trees.

In general, there was very little evidence of feeding damage on common buckthorn. The most common type of damage was leaf miner tunnels, followed by damage caused by lepidopteran larvae. Nine species were reared in the laboratory after collecting immature insects from common buckthorn indicating these nine species are able to complete their development solely on buckthorn. These included three hemipteran species, *Acanalonia conica* (Say), *M. pruinosa*, and *Gyponana quebecensis* (Provancher), three orthopterans, *Neoxabea bipunctata* (De Geer), *Oecanthus fultoni* Walker, and *Oecanthus niveus* (De Geer), and three lepidopterans collected as eggs and reared to adult, which included *Choristoneura rosaceana* (Harris), *Machimia tentoriferella* Clemens, and *Spilosoma virginica* (Fabricius). The two tortricids, *C. rosaceana* and *M. tentoriferalla* experienced high mortality during rearing and adult specimens that did emerge often had abnormal wing development. However, a literature search revealed that these nine species listed above can be categorized as generalist herbivores and are not

**Table 2.** Herbivores collected on *Rhamnus cathartica,* common buckthorn in Minnesota. Only species for which a minimum of five specimens were collected were included, except for *Oecanthus* spp., because of the high abundance of immature specimens collected.

| Order | Family | Genus species | Number of specimens | | |
|---|---|---|---|---|---|
| | | | 2004 | 2005 | Total |
| Orthoptera | Gryllidae | *Neoxabea bipunctata* (De Geer) | 10 | 5 | 15 |
| | | *Oecanthus fultoni* Walker | 2 | 1 | 3 |
| | | *Oecanthus niveus* (De Geer) | 1 | 2 | 3 |
| **Subtotal** | | | **13** | **8** | **21** |
| Hemiptera | Acanaloniidae | *Acanalonia conica* (Say) | 9 | 11 | 20 |
| | Aphididae | *Aphis glycines / nasturtii* | 0 | 39 | 39 |
| | | *Aphis glycines* Matsumura | 7 | 17 | 24 |
| | | *Aphis nasturtii* Kaltenbach | 0 | 26 | 26 |
| | Cercopidae | *Clastoptera obtusa* (Say) | 0 | 7 | 7 |
| | | *Philaenus spumarius* (L.) | 2 | 8 | 10 |
| | Cicadellidae | *Empoasca sp.* b | 0 | 5 | 5 |
| | | *Graphocephala coccinea* (Forster) | 21 | 64 | 85 |
| | | *Gyponana quebecensis* (Provancher) | 4 | 9 | 13 |
| | | *Jikradia olitorius* (Say) | 9 | 6 | 15 |
| | Derbidae | *Cedusa incisa* (Metcalf) | 8 | 2 | 10 |
| | Flatidae | *Metcalfa pruinosa* (Say) | 11 | 164 | 175 |
| | Miridae | *Hyaliodes harti* Knight | 7 | 6 | 13 |
| | | *Hyaliodes vitripennis* (Say) | 3 | 2 | 5 |
| | | *Paraproba capitata* (Van Duzee) | 6 | 8 | 14 |
| | | *Phytocoris spicatus* Knight | 2 | 4 | 6 |
| | Pentatomidae | *Euschistus tristigmus* (Say) | 8 | 9 | 17 |
| | Tingidae | *Corythucha pergandei* Heidemann | 0 | 5 | 5 |
| **Subtotal** | | | **97** | **392** | **489** |
| Coleoptera | Chrysomelidae | *Diabrotica longicornis* (Say) | 1 | 5 | 6 |
| | Curculionidae | *Polydrusus sericeus* (Schaller) | 2 | 5 | 7 |
| | Pyrochroidae | *Pedilus impressus* (Say) | 1 | 4 | 5 |
| **Subtotal** | | | **4** | **14** | **18** |
| Lepidoptera | Arctiidae | *Spilosoma virginica* (Fabricius) | 0 | 10 | 10 |
| | Gracillariidae | *Phyllonorycter caryaealbella* (Chambers) | 2 | 6 | 8 |
| | Psychidae | *Thyridopteryx ephemeraeformis* (Haworth) | 0 | 5 | 5 |
| | Tortricidae | *Choristoneura rosaceana* (Harris) | 4 | 5 | 9 |
| | | *Machimia tentoriferella* Clemens | 2 | 3 | 5 |
| **Subtotal** | | | **8** | **29** | **37** |
| Diptera | Cecidomyiidae | *Parwinnertzia notmani* Felt | 2 | 5 | 7 |
| Hymenoptera | Cynipidae | *Diplopepsis* sp. a | 0 | 9 | 9 |
| | | *Liodora* sp. | 0 | 6 | 6 |
| | Tenthredinidae | *Fenusa* sp. | 0 | 19 | 19 |
| **Subtotal** | | | **0** | **34** | **34** |

**Table 3.** Predators and parasitoids collected on *Rhamnus cathartica,* common buckthorn in Minnesota. Only species for which a minimum of five specimens were collected were included.

| Order | Family | Genus species | Number of specimens | | |
|---|---|---|---|---|---|
| | | | 2004 | 2005 | Total |
| Hemiptera | Nabidae | *Lasiomerus annulatus* (Reuter) | 10 | 15 | 25 |
| Coleoptera | Cantharidae | *Podabrus rugulosus* LeConte | 5 | 0 | 5 |
| | Coccinellidae | *Coleomegilla maculata* DeGeer | 3 | 2 | 5 |
| | | *Harmonia axyridis* (Pallas) | 44 | 68 | 112 |
| **Subtotal** | | | **52** | **70** | **122** |
| Diptera | Empididae | *Tachypeza* sp. a | 4 | 3 | 7 |
| Hymenoptera | Platygasteridae | *Leptacis* sp. c | 0 | 8 | 8 |
| | Scelionidae | *Idris* sp. | 18 | 0 | 18 |
| | | *Trissolcus* sp. a | 37 | 39 | 76 |
| | | *Trissolcus* sp. b | 0 | 38 | 38 |
| **Subtotal** | | | **55** | **85** | **140** |

considered specialist herbivores that only feed on common buckthorn.

## Discussion

Urban sites, which had the densest common buckthorn infestation and lowest plant species diversity, also had the lowest insect abundance when compared to other habitat types. All urban sites sampled were located in highly populated areas where human activities could easily disturb the natural habitat. In contrast, agricultural sites had the highest plant diversity with more insects collected at those sites. Predators were collected at higher rates in agricultural sites than the other sites possibly drawn there by agricultural pests that would be found in the adjacent crop fields.

The main objective of this study was to identify major herbivores present on common buckthorn in Minnesota. Overall, there were many herbivores collected, however; most insects collected were represented by fewer than five specimens suggesting that they were transient feeders or generalist herbivores that do not utilize common buckthorn. In reports of herbivores collected from *R. cathartica* in Europe, the most common insect species found were Lepidopterans (Malicky *et al*. 1970). Here we show that in Minnesota, defoliators were common, but unlike the situation in Europe, more Hemipterans were encountered in Minnesota than Lepidopterans. During our 2-year study we did not find any insect feeding internally on buckthorn, and thus one potential niche that could be exploited successfully would be an internal feeder such as the stem-boring beetle, *Oberea pedemontana* Chevrolat (Coleoptera: Cerambycidae) which has been identified in Europe as a possible biological control agent of *R. cathartica* (Gassmann, 2005). Even though we found many generalist herbivores feeding on leaves at no time did defoliation exceed 5% on any one tree, thus a specialist herbivore would have an abundant resource to utilize in Minnesota.

The second objective of this study was to identify possible sources of biotic resistance if non-native herbivores were introduced as classical biological control for common buckthorn. There were numerous parasitoids and predators, all considered generalists, collected from common buckthorn. The abundance of parasitoids and predators may indeed hinder establishment of potential biological control agents. Generalist predators have been known to interfere with biological control agents released for purple loosestrife control (Sebolt and Landis, 2004). Currently, there have been a few species proposed as potential biological control agents for common buckthorn in North America (Gassmann, 2005). As agent selection continues for common buckthorn, the species diversity and abundance of natural enemies collected from buckthorn and documented here should be considered. In particular, *H. axyridis* could play a significant role in preventing establishment of herbivores since it was the most abundant generalist predator collected and this coccinellid is known to prefer arboreal habitats. This recently introduced coccinellid was more common in spring and fall (Figure 1). *Harmonia axyridis* could exert strong biotic resistance on biological control agents especially if a vulnerable life stage was present when *H. axyridis* densities were high. For example, one group of candidate biological control agents for common buckthorn are the psyllids, *Cacopsylla rhamnicolla* and *Trichohermes walkeri*

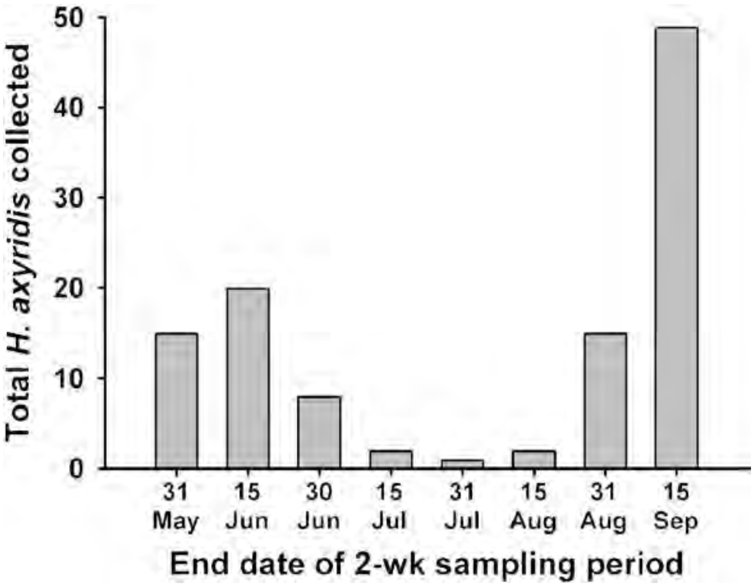


**Figure 1.** Seasonal abundance of *Harmonia axyridis* observed on *Rhamnus cathartica,* common buckthorn in Minnesota. Data pooled for 2004 and 2005.

(Gassmann 2005) and it is possible that *Harmonia axyridis* would pose a particular threat to psyllids. This potential negative interaction could be studied as part of the host testing procedure.

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# Evaluation of *Fusarium* as potential biological control against *Orobanche* on Faba bean in Tunisia

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*Summary*

A total of 149 fungal strains identified as *Fusarium* were isolated from infected *Orobanche crenata* Forsk. and *Orobanche foetida* Poir. plants. Their pathogenicity and virulence were assessed in Petri dish assays using lentils as the medium. Ten isolates were found to reduce the number of tubercles attached to the host plant. Among them, two isolates that caused necroses on tubercles of *Orobanche* in the Petri dish assays were identified as *Fusarium F6* and *F10*. They reduced the number of tubercles of *O. crenata* by 97% and 98%, respectively. Inoculums of *F6* and *F10* were produced on barley grains and were tested in sterilized and non-sterilized soil in separate pot experiments, using *O. crenata* and *O. foetida* as parasitic plants. Both isolates reduced the number of *O. crenata* and *O. foetida* by 68% and 88%, respectively, and their dry matter by 82% to 88%. A similar experiment conducted using formulated inoculums of the two isolates showed that the formulation improved the efficiency of the fungi, and reductions in the number and dry matter of tubercles to 100% were observed. These results suggest that *Fusarium* isolates have the potential to be used as biological control agents against *O. crenata and O. foetida* on faba bean in Tunisia.

***Keywords***: pathogens, pathogenicity, virulence, parasitic plants, broomrapes.

## Introduction

Broomrapes, *Orobanche* spp., of the family of *Orobanchaceae* are troublesome root parasitic weeds that cause severe damage to vegetables, legumes and sunflower (Parker and Riches, 1992). Approximately, 16 million hectares of arable land in the Mediterranean region as well as in west Asia are currently endangered by *Orobanche* infestation (Sauerborn, 1994). In Tunisia, *Orobanche crenata* Forsk. distributed in the north-east and *O. foetida* Poir. in the north-west, are the main species that cause losses in leguminous crops, especially on faba bean (Kharrat and Halila, 1994). Losses in faba bean fields can reach 80% (Kharrat, 2002). Difficulties in controlling *Orobanche* are due to the numerous tiny seeds that retain their viability in the soil for 6 to 20 years. Germination of *Orobanche* seed requires a stimulant excreted by the host plant and produces germ tubes that attach to the host plant (Raynal *et al.*, 1989). The germ tube develops a haustorium and forms a tubercle. The haustorium represents the physical and morphological contact between the parasite and the host. It supplies the parasite with water, mineral nutrients and organic materials from its host (Kroschel, 2001).

So far, no efficient control measures for *Orobanche* spp. have become available to farmers (Müller-Stöver, 2001). Single methods such as delayed sowing and use of resistant varieties have shown unsatisfactory results. The use of chemical products such as glyphosate requires care to avoid phytotoxicity. Thus, *Orobanche* represents a difficult target for selective chemical control. Control of *Orobanche* may be possible by integrating control measures. The integration of biological control with other *Orobanche* management methods is of increasing research interest. Several investigators have reported the use of fungi as potential biological agents against *Orobanche* (Waston and Waymore, 1992).

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Research has been conducted in several countries including Algeria, Egypt, Germany, Maroc and Chili (Klein *et al*., 1999; Zermane *et al*., 1999; Müller-Stöver, 2001; Boari and Vurro, 2004). *Fusarium oxysporum f.* sp. *orthoceras* (Appel and Wollenw.) Bilai obtained from diseased *O. cumana* tested in soil with sunflower as a host plant was able to reduce the number of attached and emerged broomrape seedlings by about 90% (Bedi and Donchev, 1991). *F. oxysporum f.* sp. *orthoceras* on *O. cumana* was also tested by Thomas *et al*. (1998). Recently, *F. arthroporioides* and *F. oxysporum* isolated in Israel from *O. aegyptiaca* were shown to be effective in reducing broomrape growth (Amsellem *et al*., 2001). The pathogenicity of two isolates, *Ulocladium botrytis* Preuss and *F. oxysporum* Schlecht. *f.* sp. *Orthoceras*, were tested by Müller-Stöver (2001). The two fungi cause necroses on both *O. cumana* and *O. crenata*. Currently, the development of an appropriate formulation which allows successful application of fungal propagules will determine the success of *Fusarium* in agriculture applications. The encapsulation of fungal propagules in a solid matrix 'Pesta' was used by (Müller-Stöver, 2001). A 70% reduction of *Orobanche* emergence was obtained when wheat flour kaolin granules containing chlamydospore rich biomass was applied.

Considering the importance of *O. crenata* and *O. foetida* in Tunisia and the lack of research on fungi associated to *Orobanche* spp., the main objective of this study was to screen and evaluate the potential of fungi isolated from *Orobanche* with potential as biological control agents against the parasitic weed, in laboratory and green house experiments.

# Materials and methods

## Field surveys

Field surveys were carried out from April 2004 to May 2005 in northern Tunisia, especially in the region of Nabeul. Underground stages of *Orobanche* with symptoms of fungal infections such as browning and rotting were collected. The plants were conserved in laboratory until use.

## Isolation

Isolations were made from pieces of tubercles and stems with fungal symptoms. Diseased tissues were excised, washed with distilled water, sterilized in 1% sodium hypochlorite with Tween 20 for 5 min and rinsed four to five times with sterile distilled water. After drying on filter paper, pieces were placed in Petri dishes on potato dextrose agar (PDA™) medium supplemented with 100 ppm of streptomycin. The Petri dishes were incubated in the dark at 22°C until fungal development occurred. Repeated sub-culturing was done to obtain pure cultures. Isolates were conserved on special nutrient poor agar (SNA) at 5°C for short term storage and in liquid nitrogen for long-term storage.

## Bioassays

The isolated fungi were evaluated to assess their phytotoxic ability on the growth of the underground stages of *Orobanche*. In these Petri dishes bioassays, seeds of *O. crenata* were used as the parasitic weed and those of lentil were used as the host plant. The methods followed those of Kroschel (2001). Plastic Petri dishes were filled with washed sterile sand, watered and covered with filter paper. *Orobanche crenata* seeds were sterilized with sodium hypochlorite, rinsed with distilled water and sprinkled on the filter paper at the densities of 25 seeds per square metre. The Petri dishes were covered with black plastic and incubated in the dark for conditioning at 22°C for 10 days. To enhance pre-conditioning in the *Orobanche* seed, 100 ppm of gibberellic acid was added. Pre-germinated lentil seedlings were inserted into sand in the Petri dishes through holes made in the surface of the filter paper.

To test the pathogenicity of fungal isolates, fresh colonies of the isolated fungi growing on SNA medium were used. For each Petri dish, the black plastic was removed and filter paper containing *O. crenata* seeds and lentil seedlings were sprayed with 10 ml of the spore suspension at $10^6$ spores per millilitre. The Petri dishes were incubated in the green house at 25°C and 16 h/18 h photoperiod for 5 weeks. Two replicates were used per treatment. The number of germinated, attached seeds and the number of tubercles formation were recorded.

## Test of specificity

The isolates which reduced the number of tubercles of *Orobanche* in Petri-dish assays were selected and were tested for their host specificity. A range of plants was used that included tomato (*Lycopersicon esculentum* Mill), carrot (*Daucus carota* L), Faba bean (*Vicia faba* L. and *Vicia faba* L. *minor*), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* L), wheat (*Triticum aestivum* L.) and barley (*Hodeum vulgare* L.) These plants were grown in pots in a greenhouse. Ten days after emergence roots were washed with distilled water and immersed in the inoculums at the concentration of $10^6$ spores per millilitre, for 5 to 10 min. Then plants were transplanted in pots and observed weekly for 1 month for the development of symptoms. Four plants of the same test plant species were used per pot with four pots per tested plant.

## Pot assays

Selected fungal isolates that were able to reduce *Orobanche* seed germination in the Petri dish assays were tested in sterilized and non-sterilized soils in pots in a greenhouse. Both *O. crenata* and *O. foetida* were used as parasitic weeds and *V. faba* as the host plant during this experiment.

**Preparation of inoculum:** Barely grains were used as a solid inoculum. Ten ml of sterile water were added to fresh colonies of the fungi growing on SNA medium. The resulting suspension, containing spores and mycelium, was used to inoculate the organic substrate which was then incubated for 14 days at 25°C.

**Inoculation and pot trials:** Two pot experiments, using sterilized and non sterilized soils, were conducted under greenhouse conditions to study the ability of fungi to control *O. crenata* and *O. foetida* in *V. faba* plants. In both experiments, plastic pots with 750 g capacity were filled with a mixture of soil and sand at a ratio of 2:1. *Orobanche* seeds were sprinkled onto the soil surface. The inoculum was added and mixed into the soil together with the seeds. Each pot was provided with either 11 mg of *O. crenata* seed or 9 mg of *O. foetida* seed, or 7.5 g of the solid inoculum. Three seeds of *V. Faba minor* were planted per pot and thinned afterwards to obtain one host plant per pot. The pots were fertilized and watered each week. Five controls were prepared: the host plant alone (H), the host plant with *O. crenata* or host plant with *O. foetida,* the host plant with non-inoculated substrate (H + NIS) and host plant with inoculated substrate (H + NIS). The experiments continued for 5 months and terminated when the host plant in the control was dead. The parameters used to assess the effect of fungi on the control of *O. crenata* and *O. foetida* were faba bean height (cm) and dry matter weight (g) and *Orobanche* number and dry matter weight (g).

## Formulation in pot experiments

Wheat flour kaolin granules were prepared after the methods of Connik *et al*. (1991, 1996). Durum wheat flour 32 g, kaolin 6 g and sucrose 2 g, were blended and poured into a dish. Inoculums were added as chlamydospore in 23 ml PDB (potato dextrose broth). The mixture was kneaded with gloved hands and passed through a small, hand-operated pasta maker. Obtained granules were incorporated in sterilized and non-sterilized soils. The granules were added and mixed into the soil together with the seeds. Each pot was filled with either 11 mg of *O. crenata* or 9 mg of *O. foetida* and 1 g of granules per kg of soil. Three seeds of *V. faba minor* were planted per pot and thinned afterwards to obtain one host plant per pot. In total, five pot experiments were carried out.

## Statistical analysis

All pots experiments were conducted in totally randomized design. Statistical analyses were performed using analysis of variance (ANOVA) with alpha 0.05 in GEN-STAT software.

# Results

## Field survey and isolation of fungi

One hundred and forty nine isolates were obtained from infected *O. crenata* plants collected during field surveys. All isolates were found to belong to the genus *Fusarium* after microscope examination.

## Bioassays

Ten isolates of the genus *Fusarium* reduced the germination of *O. crenata* 27% to 93% and eight reduced attachment (22% to 79%) to the host plant by germinated *Orobanche* (Table 1). The two exceptions, *F4* and *F7*, did not differ statistically between the treatments and the non-inoculated control. The number of tubercles developed by *O. crenata* was reduced by 78% to 98% (Table 1). Symptoms of necroses were observed on *Orobanche* inoculated tubercles.

Isolates *F6* and *F10* were the most efficient in reducing the percentage of tubercles of *O. crenata* by

**Table 1.** Effect of a conidial suspension of *Fusarium* on the development of the underground stages of *Orobanche crenata* in Petri dishes including; the number of *O. crenata* seeds that germinated, the number of germinating seeds that attached to the host and the number of tubercles formed.

| Isolates | Number germinated | Percent reduction in germination | Number attached | Percent reduction in attachments | Number of tubercles | Percent reduction in tubercles |
|---|---|---|---|---|---|---|
| Control | 36.5 | – | 27 | – | 47 | – |
| *F1* | 7.5 | 79 | 5.5 | 79 | 7.5 | 84 |
| *F2* | 3.5 | 90 | 18 | 33 | 10 | 78 |
| *F3* | 5 | 86 | 10.5 | 61 | 2 | 95 |
| *F4* | 3 | 92 | 29 | – | 7.5 | 84 |
| *F5* | 5 | 86 | 9 | 67 | 5 | 89 |
| *F6* | 26.5 | 27 | 13.5 | 50 | 1 | 98 |
| *F7* | 3.5 | 90 | 24.5 | 9 | 4.5 | 90 |
| *F8* | 4 | 89 | 13 | 52 | 4 | 91 |
| *F9* | 2.5 | 93 | 13.5 | 50 | 7 | 85 |
| *F10* | 23 | 37 | 21.5 | 22 | 1.5 | 97 |
| LSD(5%) | 5.74 | – | 4.03 | – | 3.5 | – |

97% and 98% respectively. These isolates were used in sterilized and non sterilized soil on *O. crenata* and *O. foetida* using faba bean as a host plant.

## Test of specificity

Isolates *F6* and *F10* were selected for use in specificity test because in preliminary tests no symptoms and no death were observed on test plant species.

## Pot assays

**Sterilized soil:** *F6* and *F10* tested in sterilized soil on *O. crenata* and *O. foetida* reduced the number germinated and dry matter weights of both parasitic plants. In *O. crenata* the number of tubercles was reduced by 70% to 87% compared to the infested the controls (H + O + NIS), and the dry matter of tubercles was reduced by 88% (Table 2). Inoculation with *F6* and *F10* isolates resulted in 36% to 38% increase in height of faba bean compared to the *Orobanche* infested control (H + IS). The dry weight of the host plant was also significantly increased by 120% to 129% compared to the *Orobanche*-infected control (Table 2).

Isolates *F6* and *F10* reduced the number of *O. foetida* by 68% to 77% whereas the dry matter was reduced by 81% to 84% compared to the infested control (H + O + NIS) (Table 2). The height and the dry matter of Faba bean was also increased by 35% to 45% and 79% to 82%, respectively, compared to the infested control (H + IS).

**Non-sterilized soils:** Isolates *F6* and *F10* incorporated with barley grains as inoculum substrate into the soil reduced the number of both *O. crenata* and *O. foetida* by more than 90% compared to the infested *Orobanche* control (Table 3). So, the dry matter of both *O. crenata* and *O. foetida* was increased by 100%, which did not statistically differ from both control of *O. crenata* and *O. foetida* (H + O + NIS) (Table 3**)**.

There were no significant effects on the height or dry matter of faba bean compared to either *Orobanche* species (Table 3). High significantly, no tubercles were produced by either species.

## Formulation experiments in pots

There were no tubercles produced and therefore no tubercle dry matter for *O. crenata* and *O. foetida* when wheat flour kaolin granules containing chlamydospore rich biomass was applied in sterilized or non sterilized soils (Figure 1).

# Discussion

The use of Petri dishes allowed observation of the underground stages of *Orobanche* (germination, attachments and tubercles) which would not have been possible

**Table 2.** Effect of isolates *F6* and *F10* on the number of tubercles and tubercle dry weight of *Orobanche crenata* and *O. foetida* and on the height and dry weight of faba bean, in sterilized soil.

| Treatments | Plant height (cm) | Percent increase | Plant dry matter (g) | Percent increase | No. of tubercle | Percent reduction | Tubercles dry matter (g) | Percent reduction |
|---|---|---|---|---|---|---|---|---|
| | *Vica faba minor,* faba bean | | | | *Orobanche crenata* | | | |
| Control (H) | 64.8 | – | 5.39 | – | – | – | – | – |
| Control (H + IS) | 65.4 | – | 5.65 | – | – | – | – | – |
| Control (H + *F6*) | 89.4 | 37 | 9.41 | 66 | – | – | – | – |
| Control (H + *F10*) | 89 | 36 | 8.6 | 52 | – | – | – | – |
| Control (H + O) | 51.4 | – | 2.66 | – | 5.2 | – | 2.02 | – |
| Control (H + O + NIS) | 53.8 | – | 2.77 | – | 4.8 | – | 1.93 | – |
| (*F6*) | 73 | 36 | 6.11 | 120 | 0.6 | 87 | 0.23 | 88 |
| (*F10*) | 74.2 | 38 | 6.35 | 129 | 1.4 | 70 | 0.25 | 88 |
| LSD (5%) | 11.33 | – | 1.81 | – | 2.18 | – | 1.14 | – |
| | *Vica faba minor,* faba bean | | | | *Orobanche foetida* | | | |
| Control (H) | 64.8 | – | 5.39 | – | – | – | – | – |
| Control (H + IS) | 65.4 | – | 5.65 | – | – | – | – | – |
| Control (H + *F6*) | 89.4 | 37 | 9.41 | 66 | – | – | – | – |
| Control (H + *F10*) | 89 | 36 | 8.6 | 52 | – | – | – | – |
| Control (H + O) | 50.2 | – | 3.32 | – | 5.6 | – | 2.12 | – |
| Control (H + O + NIS) | 54.2 | – | 3.41 | – | 4.4 | – | 2.28 | – |
| (*F6*) | 73.4 | 35 | 6.08 | 79 | 1.4 | 68 | 0.43 | 81 |
| (*F10*) | 78.8 | 45 | 6.18 | 82 | 1 | 77 | 0.36 | 84 |
| LSD (5%) | 11.33 | – | 1.81 | – | 2.18 | – | 1.14 | – |

*H* Faba bean only; *H + NIS* faba bean plus non-inoculated barley grains; *H + O* faba bean plus *Orobanche*; *H + O + F* Faba bean plus non-inoculated barley grains plus *Orobanche.*

**Table 3.** Effect of isolates *F6* and *F10* on the number of tubercles and tubercle dry weight of *Orobanche crenata* and *Orobanche foetida* and on the height and dry weight of Faba bean, in non sterilized soil.

| Treatments | Plant height (cm) | Percent increase | Plant dry weight (g) | Percent increase | No. of tubercle | Percent reduction | Tubercles dry weight (g) | Percent reduction |
|---|---|---|---|---|---|---|---|---|
| | *Vica faba minor,* faba bean | | | | *Orobanche crenata* | | | |
| Control (H) | 56.4 | – | 1.9 | – | – | – | – | – |
| Control (H + IS) | 49.8 | – | 1.8 | – | – | – | – | – |
| Control (H + *F6*) | 64 | 28 | 1 | – | – | – | – | – |
| Control (H + *F10*) | 51.8 | 4 | 1.2 | – | – | – | – | – |
| Control (H + O) | 39.8 | – | 0.8 | – | 2 | – | 0.025 | – |
| Control (H + O + NIS) | 37.6 | – | 0.9 | – | 2,2 | – | 0.025 | – |
| (*F6*) | 45.6 | 21 | 1.9 | 111 | 0 | 100 | 0 | 100 |
| (*F10*) | 45.4 | 20 | 1.6 | 77 | 0 | 100 | 0 | 100 |
| LSD (5%) | NS | – | NS | – | 0.59 | – | NS | – |
| | *Vica faba minor,* faba bean | | | | *Orobanche foetida* | | | |
| Control (H) | 56.4 | – | 1.9 | – | – | – | – | – |
| Control (H + IS) | 49.8 | – | 1.8 | – | – | – | – | – |
| Control (H + *F6*) | 64 | 28 | 1 | – | – | – | – | – |
| Control (H + *F10*) | 51.8 | 4 | 1.2 | – | – | – | – | – |
| Control (H + O) | 38.6 | – | 0.8 | – | 1.8 | – | 0.025 | – |
| Control (H + O + NIS) | 30.4 | – | 0.6 | – | 2.6 | – | 0.025 | – |
| (*F6*) | 48.4 | 59 | 1.6 | 166 | 0 | 100 | 0 | 100 |
| (*F10*) | 48.6 | 59 | 1 | 66 | 0 | 100 | 0 | 100 |
| LSD (5%) | NS | – | NS | – | 0.59 | – | NS | – |

*H* Faba bean only; *H + NIS* faba bean plus non-inoculated barley grains; *H + O* faba bean plus *Orobanche*; *H + O + F* faba bean plus non inoculated barley grains plus *Orobanche*.

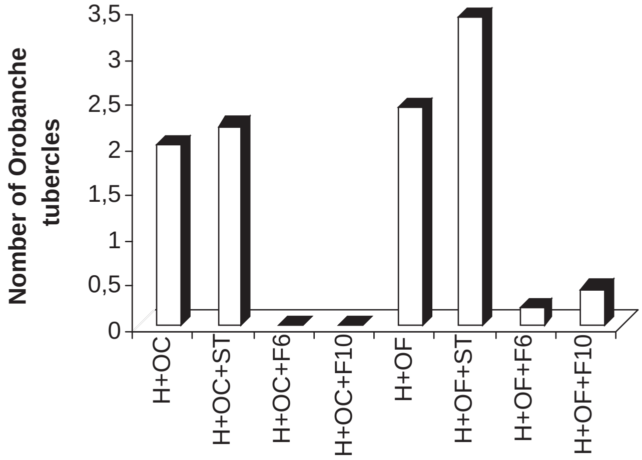


**Figure 1.** Pesta formulation effect on the number of *Orobanche crenata* and *O. foetida* tubercles.

during a pot experiment or under field conditions. In Petri dishes the fungus reduced the germination of *O. crenata* by 27% to 93% compared to the control. Thomas *et al*., (1999), found that *Fusarium oxysporum f.* sp. *ortoceras* colonized seeds of *O. cumana*, so the germination of inoculated seed was significantly reduced. Zermane *et al*. (1999) found that the germination of *O. crenata* was reduced by 50.3% when they used *F. oxysporum* in root chamber assays.

The percentage of attachment of *O. crenata* to the lentil host plant was also reduced by 79% as a result of fungus inoculum. Bedi and Donchev (1991) suggested that the black pigment of seeds protects the seed from fungal attack and they believed that the infection by the fungus occurred after seed germination. Consequently, the number of tubercles formation was significantly reduced by 98% in Petri dishes assays. Müller-Stöver (2001) found that the mortality of *O. aegyptiaca* tu-

bercles was significantly increased after inoculation in a root chamber. Accordingly, the same phenomena were also observed by Bouzoukov and Kouzmanova (1994). Thomas *et al*. (1998) suggested that *F. oxysporum* inoculated in a root chamber, attacked germination and tubercles formation. Cohen *et al*. (2002) explained the mortality process of tubercles; they suggested that the hyphen penetrated the outer cells layer within 24 h, reaching the centre of the tubercles by 48 h and infected nearly all cells by 72 h. Most of the infected tubercles had died by 96 h. We observed necroses on inoculated tubercles and the same were observed by Linke *et al*. (1992).

Of the ten isolates tested, two *F6* and *F10* were the most effective in reducing the percentage of tubercles in Petri dishes. Tests in with these two isolates in sterilized and non-sterilized soil showed that they could significantly reduce the number and dry weight of *O. crenata* and *O. foetida*. Similarly, Sauerborn *et al*. (1994) found a reduction in tubercle number of *O. cumana* parasitizing sunflower. Müller-Stöver (2001) observed a decrease of the total *O. cumana* dry matter per pot as a consequence of the application of fungi. The same phenomena were also observed by Thomas *et al*. (1998) for *O. cumana* and *F. oxysporum f. sp. orthoceras*.

Faba bean height and dry weight increased when *F6* and *F10* were used on barley grains as inoculum substrate. Zonno and Vurro (2002) using *F. oxysporum* and *F. solani* on *O. ramosa*, with tomato as the host plant, suggested that both isolates permitted growth of a larger and healthier tomato root system compared to their controls. The same reduction in numbers and dry weights of *O. crenata* and *O. foetida* were observed by us in non-sterilized soil.

These results suggested that *Fusarium* was able to grow and compete successfully with other microorganisms present in the soil (Abbasher *et al*., 1996). The use of *Fusarium* to control *O. crenata* and *O. foetida* in soil has not previously been considered for biological control. The potential of mycoherbicides for use against the parasitic plants has been investigated (Garcia Garza *et al*., 1998) and our studies indicate that this may be possible using *Fusarium* to control *Orobanche* species. Future research will be done to identify isolate *F6* and *F10* using the morphological and molecular technique.

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# Prospective biological control agents for *Nassella neesiana* in Australia and New Zealand

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Chilean needle grass (*Nassella neesiana*, Poaceae), which is native to South America, costs agriculture millions and is threatening indigenous grasslands in Australia and New Zealand. Field observations and laboratory experiments have been undertaken in Argentina to find fungal pathogens suitable as biocontrol agents. Three rust species have been selected: *Uromyces pencanus*, *Puccinia graminella* and *Puccinia nassellae*. All three have been observed causing severe damage to their host in the field and are believed to be quite host specific. Attempts to elucidate their life cycles experimentally have failed to-date, and this is discussed. *U. pencanus* is the most promising of the three because reliable methods have been developed for culturing and storing inoculum and applying it to plants. There have been some technical difficulties with the other two rusts. An isolate of *U. pencanus* has been found which can attack six of seven Australian accessions, and it has been selected for host-specificity testing. A different isolate will be needed for New Zealand populations of the weed. Mixed infections by these rusts are not uncommon in the field. Studies will continue on all three prospective candidates, as a combination may eventually be needed to achieve the desired level of control.

# Biological control of *Cirsium arvense* by using native insects

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*Cirsium arvense* is considered as one of the world's worst weeds and the third most troublesome weed in Europe. It has become increasingly problematic in ecological areas where conventional control measures are restricted. Thus control that exploits both plant competition and herbivory by specialized native insects may be an inexpensive and sustainable alternative control measure. To date, augmentation or conservation of native agents has received little attention compared to other approaches, but interest is growing. Reasons for this are that future progress in classical biological control of *C. arvense* will depend on the identification of new, host-specific herbivores from the native range and better predictions and evaluations of non-target impacts. Surveys are being conducted for herbivores on *C. arvense* in North Khorasan. We are beginning this project but already realize the importance of *Cassida rubiginosa*, a univoltine shield beetle that feeds on foliage of *C. arvense*. For successful use of this insect as a biological control agent, knowledge is required about (1) the insect densities required to obtain the desired control level and (2) the factors preventing this insect from attaining such population levels. This information may lead to the development of strategies to increase population densities of the agents.

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# The degree of polymorphism in *Puccinia punctiformis* virulence and *Cirsium arvense* resistance: implications for biological control

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*Cirsium arvense* (Californian thistle) is one of the worst weeds in New Zealand. The host-specific rust fungus, *Puccinia punctiformis*, is known to have detrimental effects on this weed; however, its usefulness for biological control in New Zealand has not been fully explored. A collection of *C. arvense* ecotypes and rust pathogen isolates from across New Zealand were used in a reciprocal interactions experiment in order to elucidate different host/pathotype infection combinations. Here, we report on the degree of polymorphism in this host/pathogen system and the possible implications for biological control.

# Field exploration for saltcedar natural enemies in Egypt

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Genus *Tamarix*, saltcedar, consists of 90 different species, and 8 of them have been introduced into the United States in the 1800s. Among them, only two species are considered a real threat to the natural ecosystems of the southwestern USA: *Tamarix parviflora* and *Tamarix ramosissima*. These weeds can be found primarily in Colorado, Utah, Kansas, Texas, New Mexico, Wyoming and Arizona (Brock, 1994; Di Tomaso, 1998). Once established, saltcedar can out-compete stressed native plants and cover large areas of formerly native habitat, resulting in a less productive and less diverse environment. Very promising results were achieved in the biological control domain by the release of the gregarious leaf beetle *Diorhabda elongata.* This work aimed to survey the entomofauna associated to *Tamarix* species in Egypt.

# The phytophagous insects associated with spotted knapweed (*Centaurea maculosa* Lam.) in northeast Romania

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Spotted knapweed is a Eurasian species that has become a problem weed, especially in mountain rangelands in North America, where approximately 7 million acres are invaded by this plant. In the second half of the past century, studies have been conducted with the purpose to introduce several natural enemies from the region of origin for the biological control of spotted knapweed. Until the present, 16 biological control agents have been introduced, of which 13 were insect species. In studies conducted in 2005 and 2006 at multiple sites in northeast of Romania, 20 insect species were obtained, belonging to the orders Lepidoptera (seven), Diptera-Brachicera (six), Coleoptera (five) and Hymenoptera-Cinipidae (two). There is an important role for species that attack new shoots in the reduction of spotted knapweed populations such as *Apion* sp. (Curculionidae), *Napomyza lateralis* (Fallen) (Diptera-Agromizidae) and Tephritidae species (Diptera) and some lepidopteran species.

# *Parkinsonia* dieback: a new association with potential for biological control

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A case study is being carried out investigating the effect of a native fungal pathogen attacking an invasive woody weed (*Parkinsonia aculeata*) in rangeland Australia. This is a new association causing impact on parkinsonia that does not appear to be occurring in its native range. Observations have shown that this dieback is capable of killing whole stands of parkinsonia in small pockets across the country. Field transects in a naturally occurring dieback site are being monitored to investigate the movement of this disease through a stand of adult parkinsonia trees. Field and glasshouse trials are being conducted to observe the effect of isolates taken from diseased plants. Trials so far indicate that two of these isolates are capable of causing disease in healthy adult plants when applied to a stem wound. Six months after inoculation, plants have been observed with large spreading stem lesions and significant reductions in plant vigour. These results are promising with potential for biological control opportunities for parkinsonia.

# Ecology, impact and biological control of the weed *Tradescantia fluminensis* in New Zealand

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*Tradescantia fluminensis* (wandering Jew, family Commelinaceae) is a serious environmental weed in many frost-free regions of New Zealand. The weed commonly forms dense, monospecific mats that completely prevent native forest regeneration. It also reduces indigenous biodiversity, alters litter decomposition and changes soil nutrient availability. Conventional control is difficult and very costly, so a biological control programme was initiated. A survey of invertebrates and plant pathogens present in New Zealand found that damage attributed to either insect herbivory or disease was minimal with little biocontrol potential. Surveys were subsequently initiated in the plant's native range in SE Brazil to identify potential agents associated with plant damage for classical biocontrol. Molecular studies are under way to determine the clonal status of the plant in New Zealand and to establish more accurately its geographic origin. In addition, the weed population dynamics will be modelled, with the aim of further understanding the ecology of this forest invader in New Zealand and improving the prospects of successful biological control.

# Potential for biological control of *Rhamnus cathartica* and *Frangula alnus* in North America

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*Rhamnus cathartica* and *Frangula alnus* are small trees of Eurasian origin, which have become invasive in North America. Some 1,000 insect samples collected at 97 buckthorn sites in Europe indicate that the insect-species richness is higher on *R. cathartica* than on *F. alnus* and includes more species that are host-specific at the species or genus level. Lepidoptera (22 species) largely dominate, followed by Hemiptera (8 species), Diptera (4 species), Acarina (4 species) and Coleoptera (1 species). Although there is no clear pattern in terms of direction of dispersal, it appears that *Rhamnus* and *Frangula* are predominant in the Old Word and New World, respectively, and this most probably explains a significant proportion of the variation in the insect-species richness on the two target plants. Minimizing potential non-target effects will likely require the selection of agents which are specific to either *R. cathartica* or *F. alnus*. There are in Europe several arthropod species which are monophagous on *R. cathartica* or the host range of which will be limited to a few species in the genus *Rhamnus*. Biological control of *F. alnus* with species- or genus-specific agents will undoubtedly be more difficult and will require additional field surveys.

# *Arundo donax* (giant reed): an invasive weed of the Rio Grande Basin

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*Arundo donax* L., giant reed, is an exotic and invasive weed of riparian habitats, irrigation canals and transportation drainages of the southwestern USA and northern Mexico. Giant reed dominates these habitats, which leads to: loss of biodiversity; catastrophic stream bank erosion; damage to bridges; increased costs for chemical and mechanical control along irrigation canals. Most importantly, this invasive weed competes for water resources in an arid region where these resources are critical to the environment, agriculture and urban users. *A. donax* is a good target for biological control because it has no close relatives in North or South America, and several insects from Mediterranean Europe are known to be monophagous. Our research program includes: (1) remote sensing and ecohydrology to determine the distribution and water use of giant reed in the Rio Grande River Basin; (2) use of microsatellites to determine the origin(s) of the invasive North American vegetative clones; (3) field studies in the native range; (4) pre-release quarantine impact studies on candidate agents, integrating ecohydrology and plant architecture to select the most promising agent(s) for full host-range testing and potential release as biological control agents.

# Potential agents from Kazakhstan for Russian Olive biocontrol in USA

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The Almaty, Kazakhstan biological control research group has been involved in Russian Olive biocontrol studies since 2006. This group has two goals: (1) to find effective biological agents (among insects) of Russian Olive and (2) to study their biological features under native conditions. Our research shows: there are about 30 insect species mentioned as strict specific natural enemies of *Elaeagnus angustifolia*: ten homopterans, two hemipterans, nine beetles, one fly and eight lepidopterans. The three most preferable potential Russian Olive biocontrol agents for introduction into the USA are one beetle and two psyllids: (1) *Altica balassogloi* Jcbs. (Coleoptera, Chrysomelidae) damages foliage and shoots, distributed in south and southeastern Kazakhstan (Arys, Ili, Karatal, Charyn rivers riparian forests); (2) *Trioza magnisetosa* Log. (Homoptera, Psylloidea), damages foliage (usually on young trees), distributed in south, central and west Kazakhstan; (3) *Trioza furcata* Low (Homoptera, Psylloidea), damages foliage (50–100% loss of foliage), distributed in central, south and west Kazakhstan. Preliminary studies indicate that the best agent for biocontrol of Russian Olive in the USA is *A. balassogloi*.

# Biology of the *Rumex* leaf defoliator sawfly *Kokujewia ectrapela* Konow (Hymenoptera: Argidae) in Urmia region

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The *Kokujewia ectrapela* Konow (Hym. Argidae) belongs to the Caspian fauna. It has been collected in Russia, Transcaucasia and Iran. The larvae of this sawfly were found living on *Rumex* spp. (Polygonaceae) and were considered as a potential biological control agent of weedy *Rumex* spp. Field surveys were conducted to determine the life history of *K. ectrapela* in Urmia region (37°31 N, 45°01 E). *K. ectrapela* completes four generations within the growing season and hibernates as a pupal stage inside the protective cocoon in the plant litter surrounding the dock plants. First generation appears from late April to end June, and the last generation appears from late August to late September. After emergence and copulation, the females attach their eggs to the margins of Rumex leaves in a single row. The average fecundity was 182 ± 15 eggs per female. Eggs hatch within 5–6 days at mean daily temperature 25°C. Young larvae begin consumption of leaves on the area in between small veins. However, once larvae are mature, they consume the entire leaf leaving only the midrib and major veins. The larvae of each generation occurred on host plants for 10–20 days depending on natural conditions. Fully grown larvae of first, second and third generations pupate within silken brownish cocoons spun using host plant material. The developmental time of *K. ectrapela* from egg to emerging adult is 25–35 days.

# What defines a host? Growth rate—the paradox revisited

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Paradoxically, plants that provide a poor diet as a defence against herbivores will potentially lose more foliage biomass to defoliators compensating by feeding longer to complete their development. It is generally believed that rapid larval growth is beneficial for the avoidance of natural enemies and that the trade-off for plants providing slow growth is the higher herbivore mortality resulting from their increased exposure to natural enemies. This argument has been found wanting in that natural enemies often prefer healthy prey from prime hosts and that herbivores may benefit from enemy-free-space by feeding on novel hosts. In fact, it appears that a slow growth rate has far more insidious effects on defoliators. Using bioassays of polyphagous Lymantriidae, a slow growth rate has been shown to correlate with the reduction of final body mass, mating opportunities, sex ratios, fecundity, dispersal abilities and pathogen resistance. Host specialization may well be driven by natural selection for improved growth rate. Unfortunately, many potential biological control agents are rejected when they feed on non-target hosts in the lab., even though they would have little chance of maintaining viable populations on those hosts.

# Selection of fungal strains for biological control of important weeds in the Krasnodar region of Russia

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Fungi, collected in different districts of the Krasnodar Region of the Russia Federation, were collected and isolated from diseased weed samples. Weeds sampled included species in the genera *Centaurea*, Salsola, *Vincetoxicum*, *Carduus*, *Cirsium,* and *Echinochloa*. Fungal isolates were selected based on biological tests and the potential of the fungi for classical and/or inundative biological control of the weeds. A live collection of plant pathogens, isolated from the collected herbarium material, was formed. This collection consists of the following genera isolated from *Centaurea solstitialis* and *Centaurea diffusa*: *Acremonium kiliense*, *Alternaria alternata*, *Alternaria radicina*, *Alternaria brassicae*, *Aspergillus*, *Cladosporium*, *Coniochaeta*, *Embellisia chlamydospora*, *Epicoccum* sp., *Fusarium culmorum*, *Fusarium heterosporum*, *Fusarium oxysporum*, *Fusarium sporotrichioides*, *Humicola* sp., *Periconia igniaria*, *Phoma sp.*, *Rhizoctonia*, *Sclerotinia*, *Sordaria*, *Ustilaginoides ochracea*, *Verticillium dahliae*. The facultative fungi *P. igniaria* E.W. Mason et M.B. Ellis (Teleomorph *Didymosphaeria igniaria* C. Booth) and *Phoma glomerata* (Corda) Wollenw. et Hochapfel. and the obligate pathogen *Puccinia hieracii* var. *hieracii* (syn. *P. jaceae*) represented the greatest interest as potential agents for biological control of *C. solstitialis*.

# Vegetative expansion and seed output of swallow-worts (*Vincetoxicum* spp.)

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Swallow-worts (*Vincetoxicum rossicum*, pale swallow-wort, and *V. nigrum*, black swallow-wort) are herbaceous, perennial, twining vines related to milkweeds (Apocynaceae). Pale swallow-wort is native to Ukraine and southeastern European Russia; black swallow-wort is native to southwestern Europe. Both species are becoming increasingly invasive in the northeastern United States and southeastern Canada. They grow in both high and low light environments in a variety of disturbed and undisturbed habitats. The success of a classical biological control program for swallow-worts will be dependent on the availability of critical biological and ecological data about the target species, such as which life stage(s) are important for population growth and most sensitive to control efforts, which in turn will affect the selection of candidate biological control agents. Assessments of the rate of vegetative expansion and reproductive output of isolated swallow-wort plants have begun at several sites in New York State, including old-field and forest understory habitats within sites. In 1 year, the number of tillers per pale swallow-wort plant increased by 45% in old fields and 19% in the forest understory. Follicle (seed pod) production was generally lower in the forest understory than old-field habitats. Monitoring will continue for at least the next 2 years.

# A new biological control program for common tansy (*Tanacetum vulgare*) in Canada and the USA

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Common tansy (*Tanacetum vulgare* L., Asteraceae) is an invasive herbaceous perennial native to Europe. It was introduced into North America as a culinary and medicinal herb. Now widely naturalized in pastures, roadsides, waste places and riparian areas across Canada and the northern USA, tansy is also spreading in forested areas. It contains several compounds toxic to humans and livestock if consumed, particularly α-thujone. Tansy reduces the productivity of pastures, displaces native vegetation in natural areas and can be a problem in regeneration of logged areas. It is listed as a noxious weed in several states and provinces. Common tansy is a good target for biological control, as it is a perennial plant growing in stable habitats and has few native North American congeners. A biological control program for common tansy is funded and coordinated by a Canadian–US consortium led by the Alberta Invasive Plant Council and the Minnesota Department of Agriculture. CABI Switzerland Centre is identifying and testing potential agents for efficacy and host specificity. Potential agents include the stem-mining weevil *Microplontus millefolii*, the leaf-feeding beetle *Cassida stigmatica*, the rhizome-mining moths *Isophrictis striatella* and *Dichrorhampha* spp., and the stem, rosette and flower head-galling gall midge *Rhopalomyia tanaceticola*.

# Surveys in Argentina for the biological control of Brazilian peppertree in the USA

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Brazilian peppertree (*Schinus terebinthifolius*: Anacardiaceae) is a perennial tree native to Argentina, Brazil and Paraguay that was introduced in the USA during the late nineteenth century as ornamental. In Hawaii, it has been mentioned as one of the most significant invasive plants, threatening federally listed endangered native plants, and in southern Florida, it has been ranked among the most important threats to biodiversity. Although most surveys have been conducted in Brazil, Argentina seems to be the most likely centre of dispersion of the genus, with 22 of the 30 species of *Schinus*. Consequently, in June 2004, an agreement for cooperation on Brazilian peppertree research was initiated between the USDA–ARS Invasive Plant Research Laboratory, Fort Lauderdale, FL, and the USDA–ARS South American Biological Control Laboratory. Recent surveys in Argentina showed that Brazilian peppertree (BP) populations harbour many phytophagous insects not previously noticed. Although most of them feed on several species within the Anacardiaceae, three of these insects, a leaf-blotcher moth, an eriophyid leaf mite and a thrips that feeds on shoots, have shown a very narrow field-host range. The potential of these natural enemies as biocontrol agents of BP, and the specificity problems implied by working in the Anacardiaceae family are discussed.

# Natural enemies of balloon vine and pompom weed in Argentina: prospects for biological control in South Africa

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As part of a new South African strategy for targeting weeds at an early stage of invasion (emerging weeds), a cooperative research agreement has been signed between the Plant Protection Research Institute (PPRI), South Africa and the USDA–ARS South American Biological Control Laboratory. The objective of this collaboration is to search for and study host-specific natural enemies of the emerging weeds, balloon vine (*Cardiospermum grandiflorum*: Sapindaceae) and pompom weed (*Campuloclinium macrocephalum*: Asteraceae). Balloon vine, a perennial woody climber, originally from tropical and sub-tropical America, and pompom weed, an ornamental herb native to South and Central America and Mexico, are considered serious invasive weeds in South Africa. Several exploratory trips were conducted in Argentina to survey for potential natural enemies of these weed species. Among the natural enemies found on balloon vine, the seed-feeding insects *Cissoanthonomus tuberculipennis* (Coleoptera: Curculionidae) and *Lisseurytomella flava* (Eulophidae) constitute the most promising candidates. Surveys on pompom weed revealed the presence of the stem-galling thrips, *Liothrips* sp. (Thysanoptera), *Cochylis* n. sp. (Tortricidae) and *Adaina* sp. prob. *simplicius* Grossbeck (Pterophoridae), two flower-feeding moths that cause considerable damage to the developing seeds. The potential of these insects as biocontrol agents is currently being assessed in Argentina and South Africa.

# *Tamarix* biocontrol in US: new biocontrol agents from Kazakhstan

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According to research of Kazakhstan biological control research group (since 1994), the four most preferable potential *Tamarix* biocontrol agents for introduction into the USA (after *Diorhabda elongata*) are: (1) the stem-galling moth, *Amblypalpis tamaricella* (Lepidoptera: Gelechiidae); (2) the foliage and flower galling psyllid,*Crastina tamaricina* (Homoptera: Psylloidea, Aphalaridae); (3) the gall midge, *Psectrosema noxium* (Diptera: Cecidomyiidae); and (4) the foliage-feeding weevil, *Coniatus steveni* (Coleoptera: Curculionidae). All can heavily damage *Tamarix* in Kazakhstan, and all have some protection from predators and from drowning. The best agent among four species is *A. tamaricella*; it inhabits riparian forests and deserts in south and southeastern Kazakhstan, and heavy infestations are capable of killing entire trees.

# Biological control of aquatic weeds by *Plectosporium alismatis*, a potential mycoherbicide in Australian rice crops: comparison of liquid culture media for their ability to produce high yields of desiccation-tolerant propagules

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In our laboratory, a methodology for the development of a stable, effective *Plectosporium alismatis* mycoherbicide is currently being investigated. In this work, we compared our standard optimized medium to other liquid media for their ability to support high conidial and chlamydospore yields and subsequent tolerance of conidia and chlamydospores to air-drying. When grown in a casamino acids-glucose based liquid medium, *P. alismatis* developed hyphae and produced high yields of conidia (1 $10^{7}$ conidia $ml^{-1}$) and dry weights (220 mg dry weights per erlen), while no chlamydospore was formed. In a nitrate-glucose based medium, growth was poor, *P. alismatis* producing aggregated hyphae that contained chlamydospores (6.5 $10^{4}$ chlamydospores per millilitre). The addition of nitrate in the casamino acids-glucose based medium restored partially chlamydospore formation (1 $10^{4}$ chlamydospores per millilitre). Although our standard, optimized medium produced 2 $10^{5}$ chlamydospores per millilitre, less than 10% chlamydospores and 50% conidia remained viable after 15 days storage at 25° , while 50% chlamydospores produced in the nitrate-glucose based medium were still viable after 30 days in the same storage conditions; moreover, these chlamydospores sporulated through a microcycle conidiation.

# Herbivorous insects from Brazil for classical biocontrol of *Tradescantia fluminensis*

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*Tradescantia fluminensis* (wandering Jew; family Commelinaceae) is a South American plant that is an exotic invasive weed in New Zealand and elsewhere. Field surveys and preliminary host range tests are underway in its native range in Brazil for herbivorous arthropods with classical biocontrol potential for New Zealand. Species found inflicting locally high levels of damage to *T. fluminensis* include two chrysomelid beetles (*Buckibrotica cinctipennis* and *Lema* sp nr *guerini*), a coleophorid moth (*Idioglossa* sp), a thrips *Scirtothrips* sp (Thripridae) and a sawfly. Other herbivorous insects located include several additional chrysomelid beetles (one a leaf-mining species), a gall midge, and the noctuid moth *Mouralia tinctoides*. This noctuid is native to South, Central and North America and appears to attack a range of plant species in the family Commelinaceae. However, the Commelinaceae (or even the order Comminales) contains no native New Zealand plant species and no plants of significant economic benefit to the country. Hence, host specificity to species, genus or even subfamily/family is, in theory, not essential in this programme. This lack of close plant relatives has also resulted in a list of plants for host-range testing that is unusual because it does not include any New Zealand native species.

# *Nigrospora oryzae*, a potential bio-control agent for Giant Parramatta Grass (*Sporobolus fertilis*) in Australia

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Giant Parramatta Grass (GPG) is an aggressive perennial tussocky grass from tropical Asia that is a declared noxious weed in Australia. It invades native pastures and reduces animal production. Its potential distribution is estimated at 23.7 million hectares in Australia. A fungus was isolated from dead shoot tips and flag leaves of *Sporobolus fertilis* (Steud.)Clayton (Poaceae) in Australia. The fungus was identified as *Nigrospora oryzae* (Berk and Broome) Petch based on the morphological characteristics and fruiting bodies. Its identity was confirmed by DNA sequencing using primers ITS 1 and ITS 4 to the internal transcribed spacer (ITS) region. *N. oryzae* was investigated as a potential bio-control agent for GPG. Forty healthy plants of uniform size were selected for the experiment. Twenty plants were inoculated to run-off with spore suspension (106/ml in 0.1% Tween 20) and the control plants with Tween 20 alone. Necrosis and cessation of growth occurred in inoculated plants but not in control plants. This is the first report of *N. oryzae* on GPG in Australia, and further trials are warranted to test its potential as a bio-control agent for this noxious weed.

# Biological control and ecology of the submerged aquatic weed *Cabomba caroliniana*

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*Cabomba caroliniana* is a submerged aquatic plant from South America that is becoming a serious weed worldwide. It spreads by seed and by fragmentation and has an extremely wide climatic range, invading lakes and ponds from tropical (Darwin, Australia: latitude 12°) to cold temperate regions (Peterborough, Canada: latitude 45°). There are currently no effective methods of managing cabomba infestations, and funding has been allocated to research biological methods. Surveys have examined cabomba in its native range and have identified several potential biological control agents. The most promising are a stem-boring weevil and two aquatic Pyralid moths. We have also examined the effects of depth and season on the dynamics of cabomba populations in Australia. We found that cabomba exhibits no clear seasonal patterns in biomass at three lakes in southeast Queensland. The plant has greatest biomass in 2–3 m depth of water (mean = 185.6 g $m^{-2}$, SD = 118.8 g $m^{-2}$), but rooted plants were found down to depths of 6 m. This study indicates that host plant resources will be available for biological control agents throughout the year, which is likely to result in more stable and potentially more effective biological control.

# Hindsight is 20/20: improved biological control of *Chromolaena odorata* (Asteraceae) for seasonally dry regions

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Currently established biocontrol agents on *Chromolaena odorata* achieve a measurable degree of control in high rainfall regions but have limited or no success in regions that experience a distinct dry, fire-prone season. Earlier consideration of this limiting factor when selecting agents may have enabled greater control than has been achieved. Insect species from seasonally dry climates within the Neotropical native range of *C. odorata* that have soil-dwelling or diapausing stages have thus become foci within the South African research programme and have relevance for control programmes elsewhere. Field host-range surveys were conducted within the native range for three potential agents, in conjunction with laboratory investigations, and for one species, with molecular and morphological taxonomic studies of specimens collected from several Asteraceae. These data provide convincing evidence regarding the unsuitability of the root-feeder *Longitarsus* sp. (Coleoptera: Chrysomelidae) and the likely suitability of the stem-galler *Conotrachelus reticulatus* (Coleoptera: Curculionidae) and stem-borer *Carmenta* sp. nov. (Lepidoptera: Sesiidae) for biological control of *C. odorata*. The value of integrating native host range investigations with laboratory studies is discussed.

# Surveys for herbivores of *Casuarina* spp. in Australia for development as biological control agents in Florida, USA

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The Australian pines, *Casuarina equisetifolia*, *Casuarina glauca* and *Casuarina cunninghamiana*, have become serious invasive weeds in southern Florida. With rapid growth and thick litter accumulation, they dramatically alter the habitat and inhibit growth of native flora and associated fauna. In coastal dunes, *C. equisetifolia* interferes in nesting of the endangered sea turtle and American crocodile. *C. glauca* occurs extensively in seasonally inundated sub-coastal areas. In addition to seed production, it suckers profusely, creating dense, ecologically barren monocultures. Despite its weed status, *C. cunninghamiana* is being considered by the citrus industry for windbreaks. Being dioecious, propagation of male plants would limit dispersal, but also have implications for biocontrol. Surveys for potential agents commenced in Australia in 2004 and includes collection of insects and galls and cones for rearing cone-feeders or granivores. Potential agents include seed-feeding and gall-forming Hymenoptera, Lepidopteran defoliators, plant-hoppers, psyllids, scales and curculionids. Many are narrowly host specific, even within the Casuarinaceae, but their potential to damage their hosts is little known, especially in the natural environment where their populations may be moderated by predators and parasitoids. Future studies aim to investigate the systematics and coevolution of insect herbivores by exploring the phylogenetic congruence between potential biocontrol agents and their plant hosts.

# Differential host preferences of *Diorhabda elongata*: implications for biological control of *Tamarix*

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In 2004–2006, releases of *Diorhabda elongata* in California resulted in poor establishment and efficacy for control of *Tamarix* spp. Previous data suggests differential host preferences favouring invasive *Tamarix ramosissima* could have contributed to establishment failure at California sites where *Tamarix parviflora* is present. In September 2006, a new population of *D. elongata* from *T. parviflora* in Greece was imported to determine if higher preference for *T. parviflora* exists and whether the program could be improved. Here, I present the results of previous experiments justifying importation of this colony. An open-field host-choice test was conducted in July 2006 between *T. ramosissima* and *T. parviflora* using the original colony. *D. elongata* showed marked ovipositional preference for mixed and *T. ramosissima* treatments over *T. parviflora* ($F = 6.57$, $df = 2{,}10$, $P = 0.015$). Adult presence was also significantly higher on these treatments than on *T. parviflora* alone (repeated measures MANOVA $F = 7.93$, $df = 2{,}14$, $P = 0.005$). A caged test conducted in Greece showed that beetles from the collection site of the original colony favour *Tamarix smyrnensis* over *T. parviflora*, whereas *T. smyrnensis* is often synonymized with *T. ramosissima* ($F = 12.66$, $df = 1{,}4.32$, $P = 0.02$). Tests will now be conducted to compare the host ranges of the new population and the original colony.

# Hybridization potential of Saltcedar leaf beetle, *Diorhabda elongata*, ecotypes

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Saltcedar (*Tamarix* spp.) is an invasive riparian shrub/tree in the western USA that displaces native plants, increases soil salinity and wildfires and lowers water tables. *Diorhabda elongata* feeds exclusively on saltcedar in Europe and Asia. The biological control potential of seven ecotypes is being tested: Fukang and Turpan, China; Chilik, Kazakhstan; Posidi and Crete, Greece; Karshi, Uzbekistan; and Tunis, Tunisia. The Fukang and Kazakhstan ecotypes are being released and defoliating large acreages of saltcedar in the northern half of the western USA, while all other ecotypes are being released in the southern half. Although different ecotypes have been released in the western USA, the effects of hybridization between ecotypes need to be understood before deliberately mixing ecotypes. All ecotypes will hybridize in controlled settings when not given a choice of mate. Some of these hybrids produce sterile offspring which could disrupt long-term population dynamics in field populations. The Greek ecotypes were tested with other ecotypes. Tests were conducted in a controlled, small caged environment, differing in sex ratio, in a controlled, larger caged environment, differing in ecotype numbers, and in a large controlled, open greenhouse. Pure-breeding and cross-breeding was observed in testing Greek ecotypes with other ecotypes. Implications for biological control will be discussed.

# Pathogens as potential classical biological control agents for alligator weed, *Alternanthera philoxeroides*

**M.G. Traversa,[1] M. Kiehr,[1] R. Delhey,[1] A.J. Sosa[2] and M.H. Julien[3]**

Alligator weed (*Alternanthera philoxeroides*) is an evergreen species native of South America. It is an invasive plant in Australia, USA, China and other countries. To identify possible candidates for the biological control of this plant, surveys of fungal pathogens were carried out in Buenos Aires and northwestern and northeastern provinces in Argentina between November 2004 and May 2005. Thirty sites were surveyed, and at least 12 fungal species were collected. *Colletotrichum orbiculare* and *Colletotrichum* cf. *capsici*, associated with stem lesions and leaf spots, were widely distributed and showed a high incidence and impact in the plant populations. *Fusarium* sp., associated with concentric large leaf spots, also had high incidence. The white rust *Albugo bliti* was collected on *A. philoxeroides* and on the closely related *Alternanthera aquatica*, but its impact seems to be limited. *Phoma* sp., *Phomopsis* sp. and other fungi have also been identified. The fungi likely to be the most promising as candidates for classical biological control of alligator weed are *Colletotrichum orbiculare*, *Colletotrichum capsici* and *Fusarium* sp.

# A survey for fungal pathogens with potential for biocontrol of exotic woody Fabaceae in Argentina

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Several exotic woody plants in the Fabaceae are aggressive invaders in native ecosystems in Argentina, especially in the Pampas. They transform local communities, replace native plant species and cause economic damage. It is assumed that a lack of natural enemies contributes to the success of these invaders. As a first step towards their eventual biocontrol, we studied the fungal pathogens naturally associated with these exotic plants in the southern Pampas region. A very aggressive dieback was observed on *Spartium junceum*, *Acacia baileyana*, *Acacia mearnsii* and less so on *Genista monspessulana*. A *Phomopsis* sp. was always found associated with this dieback. *Fusarium sacchari* var. *subglutinans* was also isolated from *S. junceum* plants showing dieback. Another *Fusarium* sp. was isolated from *A. mearnsii* plants with similar symptoms. The rust *Uromyces genistae-tinctoriae* (uredinia only), frequently infected with the hyperparasite *Sphaerellopsis filum*, was identified on *G. monspessulana*. The powdery mildew fungus *Erysiphe rayssiae* (anamorph only) was observed on *S. junceum*, where it causes some damage on re-growth. Further studies are necessary to determine whether some of these naturally occurring pathosystems could be manipulated to help control these exotic plant invaders, e. g. via the inoculative approach.

# Applied biocontrol, a landscape comparison of two Dalmatian toadflax agents

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British Columbians have a strong societal sense for protecting their environment and its resources. These values are reflected in their provincial government which has a stewardship responsibility to approximately 885,600 $km^2$ or 93% of the province (MSRM, 1997). The IAPP Application can be accessed on-line at http://www.for.gov.bc.ca/hfp/invasive/index.htm. It houses activities for management of invasive alien plants in British Columbia: planning, inventory, mechanical, chemical and biological control, the monitoring of each of these activities and biological control agent dispersal. The IAPP Application is structured to track sites and their characteristics as geographic locations. Invasive species that invade the site are then recorded. Multiple invasive species with multiple surveys can be inputted on a single site. This allows recording of the change in the invasive plant community over time as well as the level of success of our treatment efforts. A compilation of this data allows assessment of the current set of biocontrol agents in the province for a target plant species. By comparing the spread of Dalmatian toadflax to the habitat requirements of the biocontrol agents, it is possible to determine whether sufficient, suitable agents exist in the province or whether subsequent screening of agents must be pursued.

# Survey of European natural enemies of Swallow-worts (*Vincetoxicum* spp.)

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Two swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*) originating from Europe have become established in the eastern USA and Canada. Their population expansion and aggressive growth threaten native plant species, alter ecological processes and cause problems in agricultural settings. The lack of herbivory on these plants by native insects in North America and the difficulty in controlling these weeds has spawned interest in a biological control program. During 2006, surveys for potential biocontrol agents in Central and Eastern Europe revealed the herbivores: *Eumolpus asclepiadeus* and *Chrysolina aurichalcea* (Chrysomelidae); *Euphranta connexa* (Tephritidae); and *Abrostola asclepiadis* and *Hypena opulenta* (Noctuidae). Caterpillars of *H. opulenta* are leaf-feeders, and this multivoltine species successfully develops on both target weeds. This species was not previously reported developing on *Vincetoxicum*. Host- range testing has shown that both chrysomelids feed on the leaves of the target weeds as adults and the root-feeding larvae of *E. asclepiadeus* feed and develop on both target weeds. Future research will continue with host-range and specificity testing of each species to evaluate their potential as biological control agents of *Vincetoxicum*.

# Climate matching and field ecology of Australian Bluebell Creeper

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*Billardiera heterophylla* (Lindl.) L.Cayzer and Crisp (bluebell creeper) has become a serious environmental weed in Victoria, South Australia and Tasmania. Unlike its growth habit in southwest Western Australia, where bluebell creeper is indigenous, the invasive populations smother existing vegetation, out-competing and threatening local flora and fauna. Chemical and mechanical control measures have had limited success, so investigations were carried out to determine if bluebell creeper is suitable for biological control. A range of genetic and ecological experiments have been conducted on bluebell creeper to complement the surveys for natural enemies in the indigenous range. Herbivore–plant associations occurring within the invasive range were also surveyed to predict any competition against the potential biological control agents. This paper will discuss climate matching and field ecological studies that were performed. These studies were conducted to understand the differences between the indigenous and invasive bluebell creeper populations. Potential implications towards success of a biological control program and recommendations on the range of the survey area for potential biological control agents is discussed.

# Theme 4:

# Pre-release Specificity and Efficacy Testing

## Session Chair: Hariet Hinz

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# The importance of molecular tools in classical biological control of weeds: two case studies with yellow starthistle candidate biocontrol agents

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*Summary*

Molecular analyses can play a primary role in the process of host-specificity evaluation at species and population levels. In this paper, we present two examples of their application with new candidate biological control agents for yellow starthistle (YST), *Centaurea solstitialis* L. One is *Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae), a root-crown-boring weevil, which, although highly host specific, could develop on safflower in laboratory tests. Molecular genetic analyses allowed us to identify larvae from test plants in a field experiment, which conclusively demonstrated that safflower was not at risk. As a result, in 2006, the insect was petitioned for release in the USA. Another is the flea beetle, *Psylliodes chalcomera* Illiger (Coleoptera: Chrysomelidae), normally associated with *Carduus*. It was discovered feeding on YST and *Onopordum* in Russia. Laboratory experiments showed that some populations preferred different host plants. Because it was not possible to differentiate between these insect populations by morphological characters, molecular genetic analyses were conducted to infer phylogenetic relationship between these populations. Results showed a significant divergence between some populations, enabling some to qualify for further evaluation as prospective biological control agents. The ability of molecular genetics to enable us to distinguish cryptic species may greatly increase the number of potential biological control candidates available.

***Keywords***: *Ceratapion basicorne*; *Psylliodes chalcomera*; COI.

## Introduction

The main goals of classical biological control of weeds are to introduce and establish natural enemies that will effectively control pest populations and not harm non-target species. However, in relation to the former, and considering the successes achieved in the past, the outcome of a biological control programme is far from predictable. In general, only about 60% of introductions have resulted in establishments and about 30% in successful pest suppression (Crawley, 1989; Julien, 1989; Cruttwell-McFadyen, 2000). We believe that better understanding of natural enemies and pest groups will help increase rates of biological control agent introduction and success. Molecular markers can provide biological control practitioners with a valuable tool to identify cryptic species and biotypes, explore population structure and trace the origin of pests and natural

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enemies (Unruh and Woolley, 1999; Ehler *et al.*, 2004). The application of molecular methods can provide substantial help especially for insects and pathogens, where the absence of morphological distinction is frequent and accurate taxonomic identification is a key point. In addition to applied issues, molecular markers can be used to refine the phylogeny of host plants and natural enemies. Phylogenetic information is essential in understanding the evolutionary history of host plant used by insects. It provides the principal basis for the choice of the plant species to be evaluated in host-specificity testing (Wapshere, 1974; Briese, 1996).

Recently, insect molecular systematics has undergone a remarkable revolution. Advances in methods of data generation and analysis have led to the accumulation of large amounts of DNA sequence data from most of the major insect groups (Caterino *et al.*, 2000). Furthermore, the diminishing number of specialist taxonomists and the intrinsic difficulty of identifying herbivores and pathogens present a very big obstacle to biological control projects. Molecular methods, including genetic barcoding, in combination with classical taxonomy, can improve our ability to identify natural enemies (Hebert *et al.*, 2003).

The yellow starthistle (YST), *C. solstitialis* L. (Asteraceae, Cardueae), is an invasive alien weed in western USA and Canada (Maddox *et al.*, 1985; Pitcairn *et al.*, 2006). Conventional control strategies have been insufficient because of the extent of infestation and economic and environmental costs of herbicides. The weed is the target of a multidisciplinary biological control programme that involves classical and molecular approaches (Cristofaro *et al.*, 2002; De Biase *et al.*, 2003; Smith, 2004; Smith *et al.*, 2005). During the past years, two promising natural enemies for YST were identified and studied. One of these is *Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae), a root-crown-boring weevil. Although this insect was highly host specific in extensive laboratory trials, it could complete its development on safflower, *Carthamus tinctorius* L. (Asteraceae) (Smith and Drew, 2006; Smith, 2007). Therefore, open-field tests were carried out in eastern Turkey to determine if, in natural conditions, *C. basicorne* would attack safflower at locations where the insect is abundant on YST (Uygur *et al.*, 2005; Smith *et al.*, 2006). Because the insect pupates inside the plant, it was necessary to harvest plants before the adults emerged so that they could be identified. However, some plants decayed first, so we had to preserve the larvae, which cannot be identified to species based on morphology. We were able to develop a method using molecular marker systems to identify immature stages of species of the genus *Ceratapion* attacking YST and safflower in open-field conditions.

The other agent, *Psylliodes chalcomera* Illiger (Coleoptera: Chrysomelidae), is a Eurasian flea beetle, which attacks the developing stem and leaves of YST (Dunn and Rizza, 1976; Cristofaro *et al.*, 2004). *P. chalcomera* is also reported as *P. chalcomerus* by several cataloguers and specialists on flea beetles in Europe; however, we are reporting *P. chalcomera* in this paper because it is the species name historically used in the biological control of weed programme in North America (Gruev and Döberl, 1997; Campobasso *et al.*, 1999; Gruev and Döberl, 2005). In Italy, *P. chalcomera* is associated with the musk thistle, *Carduus nutans* L. (Asteraceae) (Dunn and Campobasso, 1993). Indeed, a population of the beetle from Italy was introduced in the USA to control musk thistle in 1997 (Piper and Nechols, 2004), but its establishment is unknown. More recently, two sympatric populations were found on different host plants, YST and *Onopordum acanthium* L. (Asteraceae), in southern Russia (Cristofaro *et al.*, 2004). Although Italian and Russian populations are not morphologically distinguishable, laboratory host range, life history and field experiments showed significant differences between these host-plant populations (Cristofaro *et al.*, unpublished data). In this paper, we report results of molecular analyses carried out to (1) identify the species of *Ceratapion* larvae developing in safflower and YST in field experiments in Turkey and (2) clarify the taxonomical status of populations of *Psylliodes* associated with different host plants in the field. For the two insects, we sequenced two different portions of the mitochondrial DNA (mtDNA) cytochrome oxidase I (COI). Many aspects of the structure and evolution of mtDNA have made it a valuable tool to measure genetic variation. These include its simplicity of isolation, high copy number, lack of recombination, relative degree of conservation of sequence and structure across metazoa and wide range of mutational rates in different regions of the molecule. The latter has been successfully used both in insect-population studies and species distinction (Moritz *et al.*, 1987; Wolstenholme, 1992; Simon *et al.*; 1994). These two examples show how molecular genetic tools can be used to solve critical problems in the development of new biological control agents.

# Methods and materials

## Insect specimens

A field experiment was conducted in Eastern Turkey to determine if *C. basicorne* would attack safflower at natural sites with high populations of the insect (Smith *et al.*, 2006). Larvae of *C. basicorne* develop in the upper root and lower stem of YST and pupate inside the plant. However, other species of *Ceratapion* also occurred at one of these sites. To identify the insects, we collected plants just as the insect was beginning to pupate. We kept them in individual bags until they reached the adult stage. Because many of the plants deteriorated before adults emerged, we preserved larvae in 99.9% ethanol for molecular identification. Five adults of *C. basicorne* and four of *C. scalptum* reared from experimental field plots in Eastern Turkey (Askale,

**Table 1.** Specimens of *Ceratapion* used for the genetic analysis (CB, adult *Ceratapion basicorne*; CS, adult *C. scalptum*; CON, adult *C. onopordi*; LY, larva emerged from *Centaurea solstitialis*; LC, larva emerged from *Carthamus tinctorius*).

| Species | Locality | ID no. |
|---|---|---|
| *C. basicorne* | Askale (Erzurum, Turkey) | CB2.1 |
| | Askale (Erzurum, Turkey) | CB2.6 |
| | Askale (Erzurum, Turkey) | CB3.6 |
| | Horasan (Erzurum, Turkey) | CB4.1 |
| | Askale (Erzurum, Turkey) | LY6 |
| | Askale (Erzurum, Turkey) | CB2.15 |
| *C. scalptum* | Askale (Erzurum, Turkey) | CS1.2 |
| | Askale (Erzurum, Turkey) | CS2.1 |
| | Askale (Erzurum, Turkey) | CS2.2 |
| | Askale (Erzurum, Turkey) | CS2.3 |
| | Askale (Erzurum, Turkey) | LC5 |
| | Askale (Erzurum, Turkey) | LC8 |
| | Askale (Erzurum, Turkey) | LC9 |
| | Askale (Erzurum, Turkey) | LC10 |
| *C. onopordi* | Morlupo, 31 km N of Rome, Italy | CON1 |
| | Morlupo, 31 km N of Rome, Italy | CON2 |
| | Morlupo, 31 km N of Rome, Italy | CON3 |

39°56′29.8″N, 40°35′20.1″E; Horasan, 40°7′35.9″N, 42°29′53.2″E) sites were collected and preserved in 99.9% ethanol. The adults were identified using morphology. One larva dissected from *C. solstitialis* (LY6) and four larvae dissected from safflower (labelled LC) (Askale site) were collected and preserved in 99.9% ethanol. Larval identity was afterwards confirmed by comparing their sequences to the adult sequences. Three adults of *Centaurea onopordi* were chosen as an outgroup and included in subsequent analyses (Table 1).

Genetic analyses for *P. chalcomera* were carried out on 58 morphologically identified adults killed in acetone. Over-wintering adults were collected on YST and *Onopordum* sp. in Russia (at sites near Krasnodar, 45°7′55.5″N, 36°40′29.6″E) and on *Onopordum* and *C. nutans* in Italy (at sites near L'Aquila, 42°35′N, 13°39′E, and Rome, 41°89′N, 12°50′E, respectively).

## DNA extraction, amplification and sequencing

Specimens were dried at 37°C in 1.5 ml microcentrifuge tubes, then left for 10 min at 20°C and, after the addition of 5 µl of a solution of proteinase K (20 mg/ml) with 200 µl of lysis-digestion buffer (PK buffer: 10 mM EDTA 0.5 M, 100 mM Tris 1 M pH 7.5, 300 mM NaCl 3 M, 2% sodium dodecyl sulphate), were homogenized using a pestle and incubated at 65°C for at least 3 h. The mixture was extracted with 25:24 phenol/chloroform or 25:24:1 phenol/chloroform/isoamyl alcohol protocols (Sambrook and Russell, 2000), and the DNA was precipitated in two volumes of 95% EtOH and 1/10 volume of 3 M sodium acetate. The DNA was then pelleted, washed once with 80% EtOH and resuspended in 50 µl of Tris-EDTA buffer 1×, pH 7.5. Larvae of *C. basicorne* were washed and ground in pure water. An aliquot of 2 µl of this suspension was used as the template for direct polymerase chain reaction (PCR; without preliminary DNA extraction). A part of the COI gene, 726 bp at 3′ end for *C. basicorne*, and 510 bp starting at the 5′ end for *P. chalcomera*, was amplified and sequenced using universal C1-J-2183 and TL2-N-3014 for *C. basicorne* and N2-J-1006 for *P. chalcomera* (Simon *et al.*, 1994; Antonini *et al.*, unpublished data) and specific primers [C1-SQ-2011(-)] (De Biase *et al.*, unpublished data). We used a Perkin Elmer GeneAmp PCR System 2400 thermal cycler and the following amplification conditions for both species: 95°C denaturation (1 min), 55°C annealing (30 s) and 72°C extension (1 min) for 33 cycles, followed by 7 min elongation step at 72°C. The PCR products were purified using ExoSAP-IT (©USB Corporation 2000) and sent to an external sequencing service (BMR-Genomics, Padova, Italy).

## Data analysis

All sequences were aligned by using the Staden Package v. 1.6.0 (Bonfield *et al.*, 2005). Phylogenetic analyses were performed using the software packages PAUP* v. 4.0b10 (Swofford, 2001) and MEGA v. 3.1 (Kumar *et al.*, 2004).

# Results

The COI sequence data set from the three species consisted of 726-bp aligned positions. There was almost no haplotype diversity within the population representative of each of the three species. However, a high genetic divergence (11.3%) was observed between *C. basicorne* and *C. scalptum*, which was of the same range as observed between two differentiated species *C. scalptum* and *C. onopordi* (Table 2). The tree (Figure 1) shows three clusters strongly supported at 100% bootstrapping confidences. A fragment of nearly 1000 bases was amplified for 58 individuals of *P. chalcomera*, and 510 aligned positions were used for the analyses. Tamura and Nei (1993) genetic distances (TN) were computed

**Table 2.** Genetic distances (p distances) based on COI sequence data between three species of *Ceratapion*.

| | *C. scalptum*[a] | *C. basicorne*[b] | *C. onopordi*[c] |
|---|---|---|---|
| *C. scalptum* | – | | |
| *C. basicorne* | 0.113 (0.011)[d] | – | |
| *C. onopordi* | 0.139 (0.012) | 0.144 (0.012) | – |

[a] All adults of *C. scalptum* and larvae dissected from *Carthamus tinctorius*.
[b] All adults of *C. basicorne* and larva dissected from *Centaurea solstitialis*.
[c] All adults of *C. onopordi*.
[d] Standard errors, in brackets, are estimated by bootstrap method (replications = 1000 and random number seed = 75,349)

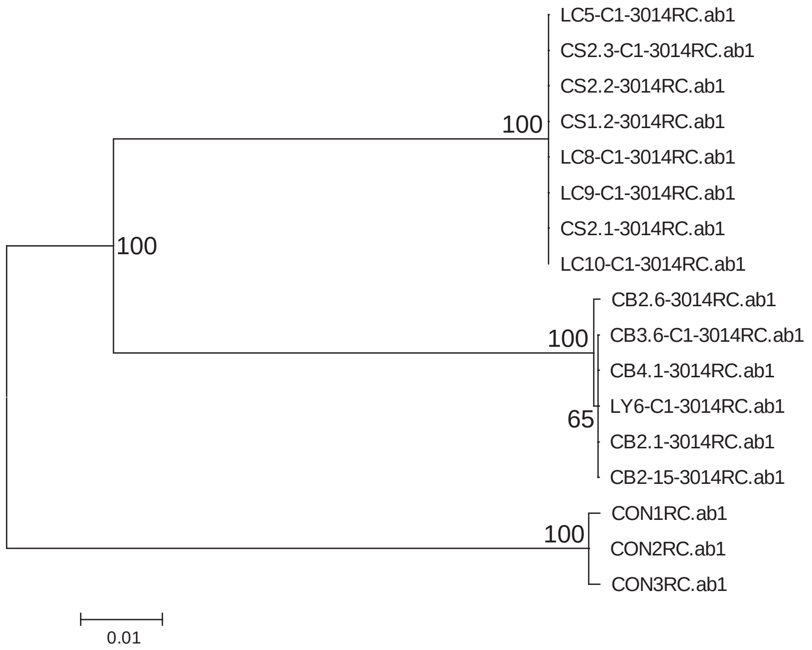


**Figure 1.** NJ tree from the analysis of COI sequence data showing phylogenetic relationships among adult and larval specimens of three *Ceratapion* species (CB, adult *C. basicorne*; CS adult *C. scalptum*; CON, adult *C. onopordi*; LY, larva emerged from *Centaurea solstitialis*; LC, larva emerged from *Carthamus tinctorius*).

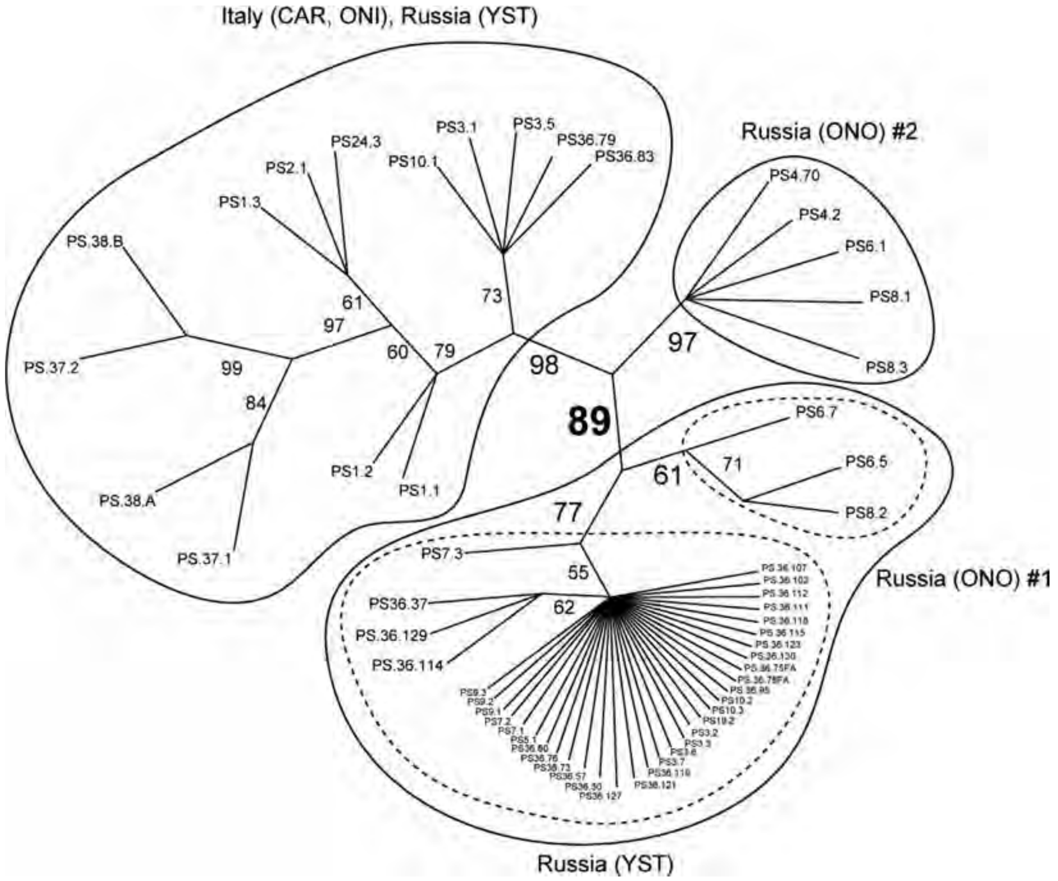


**Figure 2.** Unrooted NJ tree based on Tamura and Nei distances from COI sequences showing some differentiation of *Psylliodes chalcomera* into genetically distinct populations that are associated with different host plants [YST, yellow starthistle; ONO, *Onopordum* sp. (Scotch thistle); ONI, *O. illyricum*; CAR, *Carduus nutans* (musk thistle)].

among all pairs of sequences (table not shown; values ranged from 0.000 to 0.058). Genetic relationships among populations from different host plants and locations were inferred by tree construction using neighbour joining (NJ) of Tamura and Nei's (1993) genetic distance measure. The tree (Figure 2) shows two clusters strongly supported at 98% and 97% bootstrapping confidences. One cluster united haplotypes from Italy and Russia on YST, ONO and ONI and the second united haplotypes from Russia on ONO only. Another cluster supported at 89% confidence was formed with Russian populations from ONO and from YST.

## Discussion

*C. solstitialis* is one of the most important weeds in western USA and is a target of a classical biological control programme that started in the 1960s. Despite the introduction of several seed-head insects, the plant is not yet under control over most of its range (Pitcairn *et al.*, 2004). Recently, more effort has been placed in the discovery, selection and assessment of biological control candidates attacking the weed during early phenological stages because it is expected that stressing the immature plants may increase mortality or reduce the number of flower heads available for attack by the established flower-head insects (Smith, 2004; Uygur, 2004). *C. basicorne* and *P. chalcomera* are the most promising natural enemies attacking immature YST.

As field observations, collections and open field tests suggested the presence of sibling and closely related species for *P. chalcomera* and *C. basicorne*, respectively, we combined classical biological control strategies with the application of molecular investigations. In this paper, we have demonstrated that DNA markers are a powerful tool to better understand the system in which biological control of YST is being attempted. Molecular analyses allowed identification of immature insect stages that were recovered from YST and safflower test plants as *C. basicorne* and *C. scalptum*, respectively and exclusively. The ability to identify the species of immature insects in the field experiments was crucial in successfully evaluating the risk of introducing *C. basicorne* in the USA (Smith, 2006). Although relationship between the populations could not be definitively established in this preliminary study, the phylogeographic pattern of *P. chalcomera* s.l. gave evidence of two haplotype groups of Russian specimens from ONO and from YST. Other haplotypes that were distributed both in Italy and Russia and found on different host plants grouped together (Figure 2).

Our findings suggest different hypotheses regarding the relationships of these genetic pools. Russia (ONO) and Russia (YST) seem to be distinct gene pools well characterized and disjunct [Russia (YST) vs Russia (ONO 2)$_{TN}$ = 0.017]; however, incomplete lineage sorting or even hybridization events cannot be excluded, e.g. Russia (YST) vs Russia (ONO 1)$_{TN}$ = 0.008 and Russia (ONO 1) vs Russia (ONO 2)$_{TN}$ = 0.017. On the other hand, the structure of the first group [Italy (CAR)] of clustering individuals from two very distant locations (Russia and Italy) and feeding on three distinct host plants (YST, CAR and ONI) seems to suggest the existence of generalists shifting among different host plants. The shifted forms could eventually evolve in local specialized entities like Russia (ONO). However, mechanisms driving those shifts and their stability are still unclear.

In conclusion, available data still suggest the existence of distinct genetic entities within the *Psylliodes* spp. cf. *chalcomera* taxon, which are not distinguishable by morphological traits. Such findings seem also to reflect a trophic specialization at least at local level with two of them feeding on a single host plant (Cristofaro *et al.*, 2004). We will develop behavioral and non-lethal genetic assays to identify individuals of the Russian YST-specific population before they can be released (Fumanal *et al.*, 2005). Furthermore, because a *Carduus*-specific population has already been released in the USA, it will be important to establish if the YST-specific population can hybridize with it and to assess the effect on host specificity of the progeny. The development of microsatellites will be essential to identify hybrid individuals and male gene flow and to finally solve this interesting applied and taxonomic problem of *P. chalcomera*.

Furthermore, these two examples clearly show the importance of combining the classical biological control approach with molecular methods as a tool to discriminate between species and populations and to identify immature insects to ensure the selection of the appropriate agents. In spite of their power, the use of molecular techniques is often limited by the lack of time, costs and skills. We believe that this problem can be overcome by cooperation between groups with different expertise, and this will improve our ability to discover safe effective biological control agents. Because of the existence of cryptic species of insects, molecular genetic tools enable us to identify host-specific populations from insect 'species' previously thought to be more polyphagous. This may greatly increase the potential number of prospective biological control agents that exist in nature.

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# Fungal pathogens of *Schinus terebinthifolius* from Brazil as potential classical biological control agents

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*Summary*

The Brazilian peppertree, *Schinus terebinthifolius* Raddi, is a shrub or small tree, native to Brazil, Paraguay and Argentina. It has been introduced into other regions of the world as an ornamental or as a source of condiment. It became an aggressive invader of several exotic ecosystems, particularly in oceanic islands such as Hawaii and Mauritius, as well as in Florida, USA, and Australasia. Although research involving the use of insect natural enemies from the plant's centre of origin as biological control agents for *S. terebinthifolius* has been going on for some time, no systematic survey had been undertaken for fungal pathogens until recently. A study of the mycobiota associated with this plant was initiated in 2001, which concentrated on the southeastern states of Brazil. Eleven fungal taxa have been found thus far, several of which are new to science, namely *Hainesia lythri* (Desmaz.) Höhn., *Irenopsis* sp., *Meliola* sp., *Oidium* sp., *Phyllosticta* sp. nov., *Pleomassaria* sp. nov., *Pilidium concavum* (Desmaz.) Höhn., *Pseudocercospora* sp. nov., *Septoria* sp. nov., *Stenella* sp. and one new coelomycete genus. These fungi were associated with various symptoms, viz. leaf spots, black mildew and powdery mildew. The *Septoria* leaf-spot fungus appears to have particularly good potential as a classical biological control agent. Pathogenicity has been demonstrated to biotypes of *S. terebinthifolius* from Brazil, Florida and Hawaii. Its host range is now being tested in Florida and Hawaii with the goal of possibly introducing the fungus into those areas.

***Keywords***: classical biological control, Brazilian peppertree, surveys.

## Introduction

The Brazilian peppertree, *Schinus terebinthifolius* Raddi, known in Brazil as 'aroeira', is a small tree in the family Anacardiaceae native to Brazil, Argentina and Paraguay (Elfers, 1988; Binggeli, 1997; Taylor, 1998; Cuda *et al.*, 2006). In Brazil, *S. terebinthifolius* is found along the east coast from the state of Pernambuco in the north to Rio Grande do Sul in the south (Lorenzi and Matos, 2002). It occurs in habitats ranging from sand dunes to rainforests and semi-deciduous highland forests, often growing on river margins and in swampy areas (Binggeli, 1997).

*Schinus terebinthifolius* is generally regarded as a valuable plant in Brazil where it is used for medicinal purposes (Lorenzi and Matos, 2002) and as a source of tannin for treating fishing nets and fishing lines. The wood is used for fencing, firewood and charcoal, and the plant is also used as an ornamental and as a source of forage for goats and is valued by beekeepers (Baggio, 1988; Lorenzi, 1992). In several parts of the world, its fruits are used as a spice (pepper rosé), and there are also several other medicinal uses listed for the plant (Cuda *et al.*, 2006). However, *S. terebinthifolius* has become a noxious weed in many regions of the world where it has been introduced, such as Samoa, Fiji, French Polynesia, the Marshall Islands, New Caledonia Mauritius, and the USA, particularly Hawaii and Florida (Cronk and Fuller, 1995). It was probably introduced into Florida before 1850 as an ornamental plant (Mack, 1991). By the 1920s, it had already become widely distributed, and in the 1960s, it became recognized as an important component of the natural vegetation forming dense monocultures (Morton, 1978; Elfers, 1988; Binggeli, 1997; Anon, 2000, 2001a,b; Cuda *et al.*, 2006). Presently, the distribution of *S. terebinthifolius* in the USA includes central and

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southern Florida, southern Arizona, southern California, Texas, Louisiana, Hawaii and Puerto Rico (Anon, 2001a,b; Cuda *et al.*, 2006); it also occurs in the Bahamas (Elfers, 1988; Anon, 2001a,b). In Florida, *S. terebinthifolius* is common in areas where the soil is dry to moderately well-drained along roadsides and in the vicinity of lakes, and it is invading private and public gardens (Anon, 2002). Although it is a common pioneer of disturbed sites, such as abandoned farmland and waste areas, it is also capable of invading well-preserved natural areas, such as the drier areas of the Everglades and the coastline of peninsular Florida. Once established, *S. terebinthifolius* displaces native herbaceous communities due to its dense shading habit and the alleopathic substances it produces (Gogue *et al.*, 1974; Elfers, 1988; Binggeli, 1997; Anon, 2002; Morgan and Overholt, 2005; Cuda *et al.*, 2006). The plant is readily dispersed by birds (Lorenzi, 1992; Panetta and McKee, 1997) and is capable of vigorous regeneration after fire, cutting or frost, making its control particularly difficult (Binggeli, 1997). Invasions by *S. terebinthifolius* can result in the loss of local biodiversity (Anon, 2002; Cuda *et al.*, 2006).

Both chemical and mechanical controls of *S. terebinthifolius* have been adopted with some success in Florida and Hawaii but only in areas that are cultivated or otherwise intensively managed. Neither of those strategies is appropriate for control in environmentally sensitive natural areas, such as the Florida Everglades (Anon, 1998; Anon, 2000; Cuda *et al.*, 2006). For such areas, biological control was recognized early on as the ideal strategy for managing *S. terebithifolius*. The initial search for insect natural enemies was led by entomologists who conducted surveys in Brazil and Argentina (Hight *et al.*, 2002). Several promising insects that were found in association with *S. terebinthifolius*, including a seed-feeding bruchid beetle, a stem-boring moth and a leaf-rolling tortricid, were eventually introduced into Hawaii (Yoshioka and Markin, 1991). Later, additional natural enemies were discovered by Bennett *et al.* (1990) for possible introduction into Florida. A stem-feeding thrips, *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae), was regarded as a particularly promising candidate for biological control of *S. terebinthifolius* because it was found to be highly host-specific and damaging to the flowers and young shoots (Cuda *et al.*, 2006). Its release in Florida was approved by the US Government's Technical Advisory Group for Biological Control Agents of Weeds (TAG) in May 2007.

There are now numerous examples of the successful use of fungal pathogens as classical weed biological control agents (Charudattan, 1991; Watson, 1991; Julien and White, 1997). Surveys for fungi associated with important weeds native to Brazil, which were initiated in the mid-1990s, have yielded a plethora of fungi (e.g. Barreto and Evans, 1994, 1995a,b,c, 1998; Barreto *et al.*, 1995; Pereira and Barreto, 2005; Monteiro *et al.*, 2003; Pereira and Barreto, 2005, Pereira *et al.*, 2007; Seixas *et al.*, 2007) including many that were new to science. Two such fungi have already been introduced as biological control agents into other parts of the world, namely *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *miconiae*, which was introduced into Hawaii and French Polynesia for the control of *Miconia calvescens* D.C. (Seixas *et al.*, 2007), and *Prospodium tuberculatum* (Speg.) Arthur, which was introduced into Australia for biological control of *Lantana camara* L. (Ellison *et al.*, 2006). Until recently, no surveys of the fungi-attacking *S. terebinthifolius* in Brazil were performed, and very little information exists in the literature about this plant's mycobiota. A list of all pathogenic fungi recorded from *S. terebinthifolius* is presented in Table 1. This paper presents a preliminary account of the first survey for fungal pathogens associated

**Table 1.** Fungi recorded in association with *Schinus terebinthifolius* worldwide.

| Fungi | Distribution in association with. *S. terebinthifolius* |
|---|---|
| **Anamorphic fungi** | |
| *Alternaria* sp. | USA (Farr *et al.*, 1985) |
| *Cercospora schini* Syd. | Argentina (Chupp, 1953) |
| *Corynespora* sp. | USA (Farr *et al.*, 1985) |
| *Diplodia* sp. | USA (Farr *et al.*, 1985) |
| *Helminthosporium* sp. | USA (Farr *et al.*, 1985) |
| *Macrophoma* sp. | USA (Farr *et al.*, 1985) |
| *Phyllosticta* sp. | USA (Farr *et al.*, 1985) |
| *Sphaeropsis tumefaciens* Hedges | USA (Farr *et al.*, 1985) |
| *Verticillium albo-atrum* Reinke & Berthier | USA (Farr *et al.*, 1985) |
| **Ascomycota** | |
| *Botryosphaeria ribis* f. *achromogena* Gross. & Duggar | USA (Farr *et al.*, 1985) |
| *Botryosphaeria ribis* Gross. & Duggar | USA (Farr *et al.*, 1985) |
| *Diaporthe* sp. | USA (Farr *et al.*, 1985) |
| *Irenopsis coronata* (Speg.) F.L. Stevens | South America (Viégas, 1961) |
| *Meliola brasiliensis* Speg. | South America (Viégas, 1961; Hansford, 1961) |
| *Meliola coronata* Speg. | USA (Farr *et al.*, 1985) |
| *Nectria cinnabarina* (Tode) Fr. | USA (Farr *et al.*, 1985) |
| *Seuratia millardetii* (Racib.) Meeker | USA (Farr *et al.*,1985) |
| **Basidiomycota** | |
| *Armillaria mellea* (Vahl) P. Kumm | USA (Farr *et al.*, 1985) |
| *Armillaria tabescens* (Scop.) Emel | USA (Farr *et al.*, 1985) |
| *Ganoderma orbiforme* (Fr.) Ryvarden | South America (Viégas, 1961) |

with this plant in Brazil and an assessment of the potential of each species as a biocontrol agent for *S. terebinthifolius.*

## Materials and methods

The area surveyed for fungal pathogens of *S. terebinthifolius* covered only a small part of southeastern Brazil and was concentrated in the municipality of Viçosa and neighbouring regions in the state of Minas Gerais (MG), the municipality of Rio de Janeiro and northern coastal parts of the state of Rio de Janeiro (RJ) and parts of the valley of the Rio Paraíba do Sul in the state of São Paulo. Pathogen collections were conducted during the period from September 2001 to May 200. Although the botanical literature recognizes five varieties of *S. terebinthifolius* (Barkley, 1944), only *S. terebinthifolius* var. *acutifolius*, *S. terebinthifolius* var. *terebinthifolius*, and *S. terebinthifolius* var. *raddianus* are known to occur in the USA [only vars. *terebinthifolius* and *raddianus* occur in Florida (Cuda, 2002; Cuda *et al.*, 2006)]. Separation of such varieties in the field in Brazil was complicated by the common occurrence of intermediate forms. For practical reasons, all collections were simply referred as being from *S. terebinthifolius* without identification of host variety. Due to the difficulty in separating the varieties morphologically, the Florida populations of *S. terebinthifolius* were recently characterized genetically using microsatellites and chloroplast DNA sequence comparisons (Williams *et al.*, 2005).

Samples of diseased material were collected, dried in a plant press and taken to the lab for further examinations. Lesions were observed under a dissecting microscope and slides were prepared and mounted in lactophenol, lactophenol cotton blue or other mounting media, as required, and examined under a light microscope (Olympus BX 50) fitted with a camera and a drawing tube. Isolations were performed by either directly transferring fungal structures from sporulating lesions onto plates containing vegetable broth agar (VBA), as described by Pereira *et al.* (2003), or by transferring selected, surface-sterilized fragments of diseased tissues onto VBA plates. Pure cultures were kept in a refrigerator at 5°C in tubes containing potato–carrot agar until further use or permanent deposition in the culture collection at the Plant Pathology Department of the Universidade Federal de Viçosa (Brazil).

Pathogenicity was tested for selected fungal species either by brush-inoculating young (7-month old) healthy plants with a spore suspension adjusted to $2 \times 10^6$ spores per milliliter in a 0.05% Tween 80 solution or by depositing onto healthy plant parts 5-mm diameter culture disks cut from actively growing mycelium on VBA plates. For fungi suspected of being opportunistic wound pathogens, healthy plant parts were wounded with sterile scissors or needles before inoculation. Inoculated plants were kept in a dew chamber at 25°C for 48 h and then transferred to a greenhouse where they were examined daily for evidence of disease symptoms. Healthy plants treated in an equivalent manner but not exposed to fungal inoculum served as controls. *Schinus terebinthifolius* plants used in these tests were grown from seed originating either from Florida (Gainesville, Campus of the University of Florida), Hawaii or Brazil (Viçosa).

One fungal species collected and tested during these surveys was selected for further study. It was a species of the genus *Septoria*, which was associated with severe outbreaks of leaf spots in *S. terebinthifolius* populations, followed by extensive defoliation. A preliminary assessment of the host range of this *Septoria* sp. was performed with several local members of the Anacardiaceae: *Anacardium occidentale* L. (cashew); *Mangifera indica* L. (mango); *Schinus molle* L. (Peruvian peppertree); *Spondias lutea* L. (Brazilian plum) and *Tapirira guianensis* Aubl. (tatapiririca). Inoculations of the test plants were performed as described above.

## Results and discussion

In total, 11 fungal taxa were found attacking *S. terebinthifolius* during the surveys: three ascomycetes (*Pleomassaria* sp. possibly associated with stem dieback, *Irenopsis* sp. and *Meliola* sp. associated with black mildew symptoms); four anamorphic coelomycetes, all of which were associated with leaf spot symptoms [*H. lythri* (Desmaz.) Höhn. and *P concavum* (Desmaz.) Höhn. (synanamorphic species), *Phyllosticta* sp., *Septoria* sp. and a possible new genus of coelomycete] and four anamorphic hyphomycetes (*Oidium* sp., a powdery mildew, a *Pseudocercospora* sp. associated with leaf spots and a *Stenella* sp. associated with yellowing and premature leaf senescence; Table 2).

Eight of the 11 fungi found to be associated with *S. terebinthifolius* were isolated in pure culture. Some were extremely slow growing such as *Pleomassaria* sp., which took nearly a month to produce a visible colony, while others were relatively fast growing such as *H. lythri* and *P. concavum*. The ability of the fungi to sporulate also varied widely, but most fungi did not sporulate readily in culture. Pathogenic status was only preliminarily investigated for seven fungal species: coelomycete gen. nov., *H. lythri*, *P. concavum*, *Pseudocercospora* sp., *Septoria* sp. and *Stenella* sp. Specific details on each fungal taxon are provided below.

### *Coelomycete* gen. nov.

This fungus was compared with a series of possible genera of other coelomycetes to which it resembled but did not match any of them. We concluded that it represents a new fungal genus that will be described in a separate publication on the taxonomy of the fungi on *S. terebinthifolius*. This new fungus was restricted

**Table 2.** Synopsis of observations on fungi collected on *Schinus terebinthifolius* during surveys in Brazil.

| Fungus | Disease | Damage to host | Purported specificity | Culturability | Biocontrol potential |
|---|---|---|---|---|---|
| Coelomycete (gen nov.) | Leaf spot | Moderate | Uncertain | Cultivable | Uncertain |
| *Hainesia lythri* | Leaf spot | Insignificant | Non-specific | Cultivable | None |
| *Irenopsis* sp. | Black mildew | Insignificant | High | Not cultivable | None |
| *Meliola* sp. | Black mildew | Insignificant | High | Not cultivable | None |
| *Oidium* sp. | Powdery mildew | Moderate | High | Not cultivable | Moderate |
| *Phyllosticta* sp. | Leaf spot | Moderate | Uncertain | Cultivable | Uncertain |
| *Pleomassaria* sp. | Branch dieback | Uncertain | Uncertain | Cultivable | Uncertain |
| *Pilidium concavum* | Leaf spot | Insignificant | Non-specific | Cultivable | None |
| *Pseudocercospora* sp. | Leaf spot | Severe | High | Cultivable | None (see comments) |
| *Septoria* sp. | Leaf spot | Severe | High | Cultivable | Very high |
| *Stenella* sp. | Leaf spot | Moderate | High | Cultivable | Uncertain |

in its distribution, being found in only one location on plants growing on a hill in a coastal sand dune area in the state of Rio de Janeiro. It was always associated with a very distinctive circular leaf spot, and the disease was regarded as severe on the plants it was attacking, resulting in significant defoliation. Its pathogenicity was proven by Koch's postulates, but disease levels obtained under controlled conditions were less severe than those observed in the field. Perhaps, the fungus requires particular environmental conditions, such as those occurring where it was repeatedly collected, to become epidemic and to cause severe disease levels. It is difficult to determine at this stage whether this fungus has any potential as a classical biological control agent.

## *H. lythri* and *P. concavum*

These fungi are known to be opportunistic pathogens, depending on wounds for infection; this study confirmed this relationship. Only wounding of tissues before inoculation allowed for disease to develop during the pathogenicity tests. These two species are known to be generalists having been described from a wide range of hosts (Shear and Dodge, 1921; Palm, 1991). Among these are several species belonging to the Anacardiaceae, e.g., *Rhus glabra* L., *Rhus typhina* L., *Toxicodendrun radicans* (L.) Kuntze and *Rhus aromatica* Ait. (Greene, 1950). This information alone would make these fungi of little interest for classical biological control because they might pose a threat to native plants in the area where introduction would take place. *P. concavum* has been frequently reported in association with old lesions of *H. lythri*, but the two species are normally not found simultaneously on the same lesions or during the same season. However, this association is not observed in all hosts (Shear and Dodge, 1921; Palm, 1991). In one study, *H. lythri* and *P. concavum* were found occurring together during the summer, but it is interesting to note that *P. concavum* was associated with non-senescent leaves; this suggests that, in the case of *S. terebinthifolius*, *P. concavum* may act as a true pathogen. Isolations of *H. lythri* from diseased tissues resulted in typical *H. lythri* colonies in pure culture, while isolations of *P. concavum* also resulted in typical *Hainesia* colonies in culture. This has already been reported for isolates from other hosts (Shear and Dodge, 1921; Palm, 1991), and it is accepted that *H. lythri* and *P. concavum* are genetically connected by being synanamorphs of the same ascomycete species, *Discohainesia oenotherae* (Cooke & Ellis) Nannf. (Palm, 1991). Although interesting and a new report for *S. terebinthifolius*, these two records have no significance for biological control.

## *Irenopsis* sp. and *Meliola* sp.

These are two ascomycetes belonging to the family Meliolaceae, a family of fungi that is widely distributed in the tropics and contains highly host-specific, obligate parasites that cause diseases known as black mildews (Hansford, 1961; Kirk *et al.*, 2001). *Irenopsis* sp. and *Meliola* sp. often were found attacking the same *S. terebinthifolius* individuals in the field and sometimes occurring together on the same leaf. Their colonies were indistinguishable with the naked eye. A comparison of the morphology of these fungi with known black mildew species associated with the Anacardiaceae led to the conclusion that both species on *S. terebinthifolius* are new to science. These will be described in a separate publication. Although pathogenic, diseases caused by the Meliolaceae are generally considered to be too weak to be of any interest for use in weed biological control.

## *Oidium* sp.

This fungus is an anamorphic form from the Erysiphaceae, an important family of ascomycetes that are

obligate biotrophs causing powdery mildews. Fungi in this group can be highly host-specific and cause heavy losses in some crop plantations. Unfortunately, in the case of this fungus, it was found only on a few occasions, always during the dry and colder season of the year, and damage to *S. terebinthifolius* was regarded as only moderate. Perhaps, further studies will reveal more aggressive strains of this fungus that may be of interest to be used in classical introductions. The complete identity of this fungus remains obscure at this stage. A preliminary DNA analysis surprisingly placed this taxon close to *Oidium tuckeri* Berk., the aetiological agent of the powdery mildew of grapes, than to the Erysiphales known to attack the Anacardiaceae.

## *Phyllosticta* sp.

*Phyllosticta* is a genus that contains some important pathogens of crop plants (Nag Raj, 1992), but there also are saprophytes and endophytes in this genus. The species of *Phyllosticta* recorded on the Anacardiaceae are difficult to separate taxonomically and are, in general, poorly described in earlier publications. These are *Phyllosticta schini* Thüm, *Phyllosticta rhois* West., *Phyllosticta rhoina* Kalch., *Phyllosticta toxicodendri* Thüm and *Phyllosticta toxica* Ell. & G. Martin (Saccardo, 1884, 1902). A precise comparison with the fungus found on *S. terebinthifolius*, based on such descriptions, was impossible. A review of the genus was published by van der Aa and Vanev (2002), and these authors considered that all the taxa listed above, except *P. toxica*, should be excluded from *Phyllosticta*. It is likely that the species collected in this survey represents a new taxon for the genus, but further investigation is required to confirm this. Obtaining an isolate of *Phyllosticta* sp. from *S. terebinthifolius* proved difficult. An isolate was finally obtained but in the latter stages of this work, which did not allow for confirmation of its pathogenicity. The leaf-spot disease on *S. terebinthifolius* with which *Phyllosticta* sp. was consistently associated was often severe. Further studies on this fungus as a possible biological control agent should be given high priority.

## *Pleomassaria* sp.

This fungus is a new species for this ascomycete genus, which will be described separately. Its pathogenic status is still uncertain. The fungus was found only once during examinations of branches showing dieback symptoms (in Guaraciaba, state of Minas Gerais). Such diebacks are very commonly observed in the field in Brazil and are often very debilitating to the host plants. We have observed that most of the striking differences between healthy versus unhealthy plants when they are growing in exotic situations, such as in Florida, compared to plants from the native range, such as in Brazil, are the result of this dieback of a large proportion of branches, particularly the lower ones. The aetiology of this disease has thus far been elusive. Isolations from inner parts of diseased branches usually yield a range of sterile fungi, which do not yield disease symptoms when inoculated onto healthy plants. *Pleomassaria* was thought to perhaps be the aetiological agent involved in such diebacks. The fungus was isolated in pure culture but grew extremely slowly and did not sporulate. Nevertheless, work on this fungus should be continued to clarify its possible involvement in this spectacular disease.

## *Pseudocercospora* sp.

Until this study, there were no members of the genus *Pseudocercospora* known to infect *S. terebinthifolius* or any other species in this genus. We found two kinds of *Pseudocercospora* spp. associated with leaf spots on *S. terebinthifolius*. They have distinct morphological features and may deserve to be treated as separate species, to be named and described in a separate publication. In terms of disease symptoms produced by these two species on *S. terebinthifolius*, they were essentially indistinguishable. Pathogenicity to *S. terebinthifolius* was demonstrated for one isolate. Because isolates of the two fungi did not sporulate in culture, Koch's postulates were performed with culture disks serving as inoculum. Disease symptoms produced with this method were not very severe and were different from those observed in the field. *Pseudocercospora* leaf spot was one of the most common diseases of *S. terebinthifolius* in the surveyed areas in Brazil and sometimes led to significant levels of defoliation. Unfortunately, it appears that *Pseudocercospora* spp. have no potential for classical biological control, as on two separate visits to Florida, one of us (RWB) collected leaf spots in the Everglades and near the town of Plantation, bearing *Pseudocercospora* colonies. Unless an especially virulent *Pseudocercospora* strain is obtained from Brazil, introductions of this fungus would probably be superfluous and innocuous, at least in Florida, as the fungus is already present in areas where S. *terebinthifolius* is a problem but is not reducing infestations.

## *Septoria* sp.

This fungus was compared with other members of the genus *Septoria* described in the literature on members of the Anacardiaceae and is clearly distinct. It represents another new taxon discovered during this survey to be fully described in a separate publication. Although it grew slowly, *Septoria* sp. sporulated abundantly on VBA. Its pathogenicity to *S. terebinthifolius* was demonstrated, and abundant lesions formed and coalesced leading to substantial defoliation of inoculated plants. A preliminary host-range study performed with this fungus indicated that it is highly host-specific, which is often observed for other members of

*Septoria* as discussed in the review by Priest (2006). Fungi in this genus already have been used for classical biological control of weeds. Three examples involved introductions of species of *Septoria* into the USA, all coincidentally in Hawaii. *Septoria passiflorae* Syd. was introduced from Colombia in 1995 for biological control of *Passiflora tarminiana* Coopens (=*Passiflora mollissima*, *Passiflora tripartita*, *P. tripartita* var. *tripartita*) (Norman, 1995). Another *Septoria* sp. was introduced from Ecuador as a biological control agent against *L. camara* L. in 1993 (Trujillo and Norman, 1995). *Septoria hodgesii* Gardner was regarded by Gardner (1999) to have potential for biological control of *Myrica faya* (Ailton) Wilbur. It was also introduced in Hawaii in 1997 but did not establish, probably due to unsuitable environmental conditions at the release sites (E. Killore, personal communication). Excellent control of *L. camara* and *P. tarminiana* was reported after the introductions of *Septoria* spp. against these weeds (Trujillo, 2005). Likewise, *Septoria* sp. collected on *S. terebinthifolius* appears to have good potential as a classical biological control agent. It not only caused considerable damage through defoliation of infected plants in the field in Brazil but was found to be pathogenic to plants grown from seeds of *S. terebinthifolius* from Hawaii and Florida. More importantly, it appears to be host-specific, as it did not infect any of the other five species of Anacardiaceae (i.e. cashew, mango, Peruvian peppertree, Brazilian plum and tatapiririca) included in the preliminary host-range test performed during this study. Isolates of this fungus are now under additional evaluation in approved quarantine laboratories located in Hawaii (HDOA-Biological Control Labs, Honolulu) and in Florida (FLDACS, DPI Pathogen Containment Laboratory, Gainesville).

### *Stenella* sp.

Most members of the genus *Stenella* are plant pathogens causing leaf-spot diseases. There are over 20 species described in the literature (Kirk *et al.*, 2001), but none was described in association with *S. terebinthifolius* or any other member of the Anacardiaceae. This fungus appears to be a new taxon of cercosporoid fungus, also to be described later. Observations in the field strongly indicated that this is a pathogenic fungus that forms extensive brown colonies on adaxial leaf surfaces, accompanied by abaxial yellowing and premature dropping of infected leaves. Unfortunately, pathogenicity was not proven during attempts to fulfil Koch's postulates. One possibility is that the use of culture disks of this fungus as inoculum was inadequate for that purpose or that an incompatible combination of fungal isolate and host genotype led to such a failure. This is, therefore, still considered in this study as an unresolved issue, and the subject of its potential for biological control of *S. terebinthifolius* will be pursued in a subsequent study.

The list of fungi already recorded in association with *S. terebinthifolius* (Table 1) contained 16 records from the USA, but only four from the centre of origin of the plant in the Neotropics. This survey, although preliminary and covering a small part of the native distribution of *S. terebinthifolius*, raised the number of fungi known to attack this species in the Neotropics to 14. This type of result is not uncommon and was already observed for other weeds native to Brazil such as *L. camara* (Barreto *et al.*, 1995) and *Chromolaena odorata* (L.) R.M. King & H. Rob. (Barreto and Evans, 1994). After becoming invasive in new, exotic situations, these plants become ubiquitous and an abundant substrate for saprophytic or generalist fungi. Fungal collections become frequent, and many fungal–host associations are then described and published. The majority of the records from the USA are probably explained by this approach. For instance, fungal species such as *Armillaria mellea*, *Armillaria tabescens*, *Ganoderma orbiforme*, *Botryosphaeria ribis*, and *Nectria cinnabarina* (Table 1) are well-known generalist pathogens. Conversely, fungi already recorded from the Neotropics or those newly recorded in this study are (with the clear exception of *H. lythri* and *P. concavum*) likely to be specialized host-specific pathogens.

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# Testing the efficacy of specialist herbivores to control *Lepidium draba* in combination with different management practices

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*Summary*

*Lepidium draba* L. [=*Cardaria draba* (L.) Desv.; Brassicaceae] is a perennial mustard, indigenous to Eurasia. In the 18th century, *L. draba* was introduced into North America, where it is now listed as noxious in 16 states and three Canadian provinces. In 2001, a biological control program was initiated investigating the possibilities for biological control of *L. draba* in North America. To determine whether specialist herbivores are actually limiting population growth of *L. draba* in its area of origin and how their effect might interact with soil nutrients and management regimes, we established manipulative field experiments in Spring 2006 in eastern Romania, where five of the currently studied biological control agents are present. Plots (3 × 3 m) were established in already existing *L. draba* stands, and four treatments applied in a split-plot design: (1) grazing (yes, no); (2) cultivation (none, shallow, shallow + sowing of grasses/legume mix; (3) pesticide application to exclude herbivores and/or augmentation of specialists (yes, no) and (4) carbon addition in the form of sawdust to reduce plant available soil nitrogen (yes, no). First results indicate that cultivation and cultivation + sowing reduced the number of *L. draba* plants, however only at the beginning of the field season. As expected, neither pesticide nor sawdust applications had an effect on plant numbers or plant vigor in the first year; they will probably need more time to become apparent. We expect that grazing treatments, which will start in May 2007, will probably have the largest effect on *L. draba* vigor and densities. Final results should allow us to develop recommendations for an integrated management strategy for *L. draba*.

***Keywords***: integrated weed management, top–down effects, hoary cress, grazing, carbon addition, insect exclusion.

## Introduction

Hoary cresses or whitetops, *Lepidium* spp. (=*Cardaria* spp.), are perennial mustards of Eurasian origin (Hegi, 1987), which were introduced to the USA in the late 19th century and have since then spread throughout the western and the northeastern states. They are aggressive invaders of crops, rangelands and riparian areas, but they grow particularly well in disturbed and/or irrigated areas (Lyons, 1998). Because they are difficult to control sustainably using mechanical or chemical methods, a consortium was established in Spring 2001 to investigate the scope for classical biological control. As a result of literature and field surveys conducted at CABI Europe–Switzerland between 2001 and 2003, seven phytophagous insect species were prioritized as potential biological control agents based on records of their restricted host range, and five species (four weevils and one flea beetle) are currently being investigated (Cripps *et al.*, 2005). In addition, one gall-forming weevil and an eriophyid mite are being studied by the US Department of Agriculture Agricultural Research Service and European Biological Control Laboratory in Montpellier, France, and Montana State University, Montana, respectively.

One of the assumptions in biological weed control is that, in their area of origin, invasive plants are regulated by natural enemies. However, evidence for the top–down regulative ability of herbivores is equivocal (Crawley, 1989; Price, 1992; Maron and Vilà, 2001 and references therein). The outcome of studies depended

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on the life history of the plant species investigated, the herbivore community and on environmental conditions (Brown and Gange, 1989; Louda and Potvin, 1995; Maron *et al.*, 2002; DeWalt *et al.*, 2004).

To investigate whether herbivory, particularly by specialists, has an effect on *Lepidium draba* at the population level, we established two identical large-scale experiments at two natural field sites of *L. draba* in eastern Romania, where five of the currently studied potential biological control agents occur. These include the gall-inducing weevils, *Ceutorhynchus cardariae* Korotyaev (Col., Curculionidae) and *Ceutorhynchus assimilis* (Paykull) [=*C. pleurostigma* (Marsham)], the seed-feeding weevil, *Ceutorhynchus turbatus* Schultze, the stem-mining flea beetle, *Psylliodes wrasei* Leonardi & Arnold (Col., Chrysomelidae) and the gall mite, *Aceria drabae* (Nal.) (Acari, Eriophyidae). Results will indicate whether herbivores are actually limiting population growth of *L. draba* in its area of origin and how their effect might interact with soil nutrients and management regimes. This should ultimately help us to develop an integrated management strategy for *L. draba*.

## Materials and methods

### The study species

*L. draba* is a herbaceous, long-lived perennial that reproduces vegetatively and by seed (Lyons, 1998). In the introduced range, established plants bolt in early spring, flower in May and June and set seed in July. A combination of several control practices, including herbicide application and physical removal by hoeing or tilling, followed by plantings of competitive species is considered the most effective control strategy for *L. draba* (Lyons, 1998). Sheep will graze *L. draba* and especially like seedlings (Lyons, 1998). However, a complete grazing management program has not been developed yet (Sheley and Stivers, 1999).

Females of *C. cardariae* lay their eggs in the leaf midribs, petioles and developing shoots of *L. draba* from early spring until mid-June (Hinz *et al.*, 2007). Female oviposition induces the galls, in which the larvae mine and develop. The root-gall-forming weevil, *C. assimilis*, has several generations per year (Jourdheuil, 1963) and appears to have at least two different host races, one of which may be host-specific to *L. draba* (Fumanal *et al.*, 2004). The seed-feeding-weevil, *C. turbatus*, lays its eggs into the developing fruits of hoary cress and its larvae destroy one or both seeds during their development. *P. wrasei* lays its eggs between the end of August and the end of October in the soil close to shoots of *L. draba*. After an obligate diapause, larvae hatch from eggs the following spring and feed in the developing shoots and vegetative points of *L. draba* (Hinz *et al.*, 2007). *C. cardariae*, *C. assimilis* and *P. wrasei* can kill whole ramets or developing shoots of *L. draba* or at least stunt their growth (Fumanal *et al.*, 2004; Hinz *et al.*, 2006). The gall mite, *Aceria drabae* (Nal.) (Acari, Eriophyidae), overwinters in dormant shoot buds of *L. draba*. In spring, the mite is passively carried up in the developing shoot where it feeds on meristematic tissue (J. Littlefield, personal communication). The wind-dispersed mites have several generations per year and can reduce or completely prevent seed production (Lipa, 1978). Extensive host-specificity tests have shown that both *C. cardariae* and *A. drabae* have a narrow host range, and petitions for field release of both species will start to be prepared in Autumn 2007.

### The field sites

Experiments were established at two natural field sites of *L. draba* situated in Eastern Romania, which forms part of the area of origin of *L. draba* (Hegi, 1987). One is near Iaşi (47°10′N, 27°27′E) and is about 100 ha; it is the largest site of *L. draba* we found during our surveys. The site is used as a pasture for peasants' cattle and horses from the neighbouring villages and is heavily overgrazed. We believe that cattle avoid grazing *L. draba* and, in combination with the disturbance caused by the livestock, this is assumed to favor *L. draba* and may have facilitated its increase to the exceptional current population.

The second site is a sheep pasture of about 70 ha and belongs to the Central Research Station for Soil Erosion Control in Perieni (48°16′N, 27°38′E), about 100 km south of Iaşi. *L. draba* occurs in localized areas, about 0.5–1 ha in size.

### The field experiment

At the end of March 2006, six blocks (18 × 22 m) were established at each of the two field sites. Twelve plots (3 × 3 m), separated by 2-m buffer zones, were set up within each block (i.e. 72 in total per site), and the following four treatments were applied in a split-plot design: (1) grazing (yes, no); (2) cultivation (none, shallow, shallow + sowing of grass/legume mix); (3) pesticide application to exclude herbivores and/or augmentation of specialists (yes, no) and (4) carbon addition in the form of sawdust to reduce plant available soil nitrogen (yes, no). On 29 March 2006, plots were harrowed, and on 1 April, a grass/legume mixture was sown according to treatments. At the beginning of May, all blocks were fenced to protect plots against grazing animals to allow the newly sown grass and disturbed plants in cultivated plots to recover. From the beginning of May 2007 onwards, one side of half of the plots is opened for 1 to 2 weeks per month to either cattle and horses (Iasi) or sheep (Perieni).

To exclude phytophagous arthropods, the systemic insecticide imidacloprid (Confidor 200; Bayer AG,

Leverkusen, Germany) and the miticide abamectin (Vermitec 18; Agri-Mek™, 0.15 EC, Syngenta SA, Basel, Switzerland) were applied as a soil drench in the second half of April 2006 at a rate of 13.5 and 1 ml, respectively, per plot (3 × 3 m). Thereafter, plots were sprayed in 2-week intervals until July with a mixture of abamectin and the pyrethroid cypermethrin (Cyperguard 25 EC; Gharda Chemicals Ltd., India) at a rate of 1 and 0.2 ml, respectively, per plot. Because abamectin did not work satisfactorily, it was later replaced by hexythiazox (Nissorun 10 WP; Nippon Soda Co., Ltd., Japan) at a rate of 0.5 g per plot. All pesticides applied were tested for their potential direct effect on growth of *L. draba*. Plots not receiving pesticides were sprayed with an equal volume of water. For plots with carbon addition, sawdust was applied once a month between April and June by hand to the soil surface at a rate of 1.5 kg/m$^2$ per year.

On 19 May and on 7 July, experimental plots in Perieni were infested with *A. draba* by collecting *Aceria*-damaged *L. draba* plants in the vicinity of Iasi and placing them close to *L. draba* plants on the plots not treated with pesticides. In addition, about 50 individuals of four of the potential agents (i.e. *C. assimilis*, *C. cardariae*, *C. turbatus*, *P. wrasei*) and other weevils and flea beetles associated with *L. draba* were released on each non-treated plot in Perieni at the end of June. In Iasi, nearly all plants were naturally attacked, so no artificial infestations were made.

In each 3 × 3 m plot, smaller 0.5 × 0.5 m subplots were established during April, and the number of *L. draba* plants (ramets) was recorded four to five times between mid-April and mid-July 2006. During May, plant traits (i.e. phenological stage, number of shoots and height of each shoot) were recorded for a maximum of 20 plants per subplot, chosen along two diagonal lines. In addition, any foliage damage (visible from the outside) was noted and, as far as possible, attributed to specific herbivore species. To record potential differences in species composition, visual ground cover estimates were noted in three 0.5 × 0.5 m subplots during July, and the following categories were recorded: percent cover of *L. draba*, forbs, legumes, grasses and bare ground.

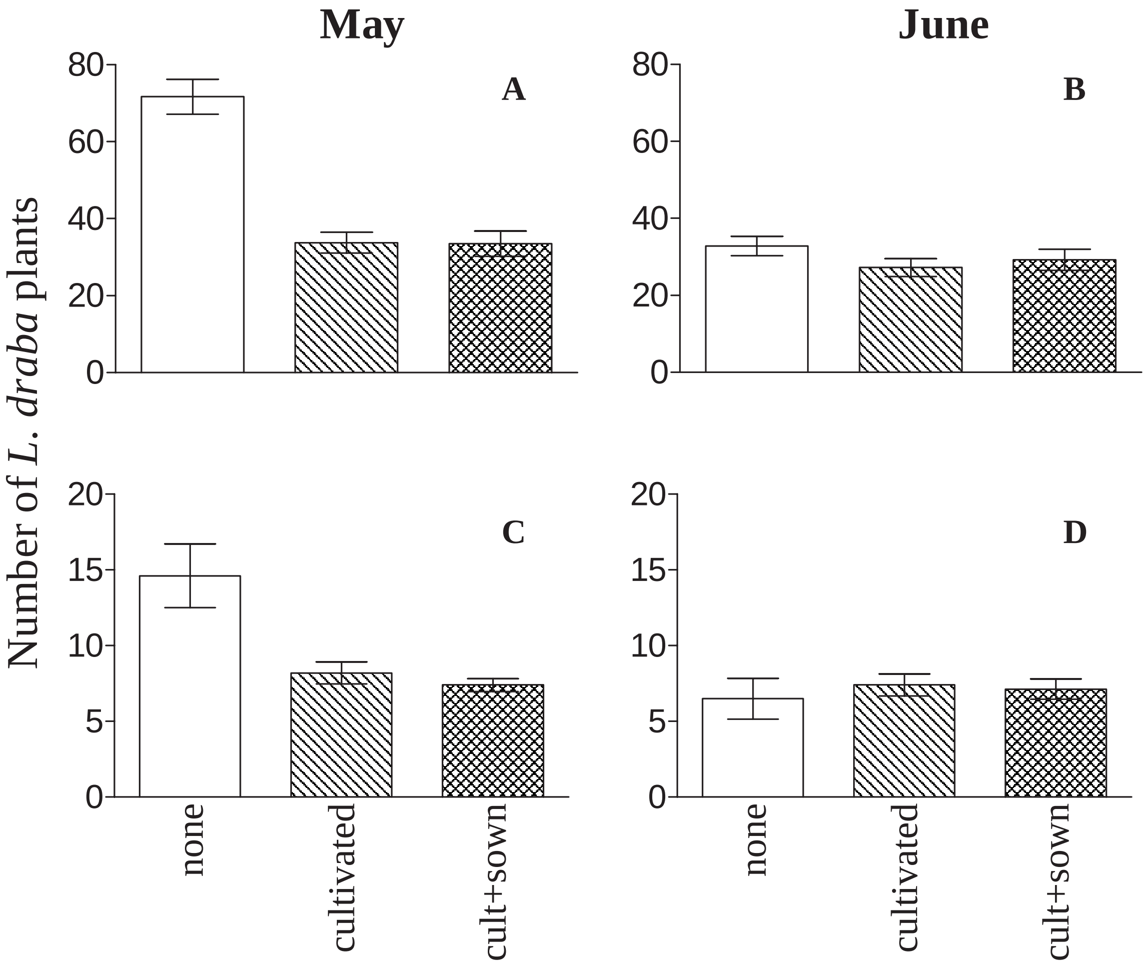


**Figure 1.** Mean number (±SE) of *Lepidium draba* plants in 0.25 m$^2$ quadrats in May and June 2006 at Iasi (a and b) and Perieni (c and d) after different cultivation regimes.

Plant traits were analysed using a hierarchical design with pesticide application (yes, no) and sawdust addition (yes, no) nested within cultivation treatments. Because grazing treatments will only start in Spring 2007, they were not included in the analyses of the 2006 data. Plant numbers were analysed using repeated measures analysis of variance. Data on percent cover and percent plants reproducing were arcsin-transformed. All analyses were conducted with Statistical Package for the Social Sciences 14.0.

## Results and discussion

The density of *L. draba* was four to five times higher in Iasi than in Perieni (Fig. 1), presumably because of the long history of stronger grazing pressure on the associated vegetation in Iasi. Cultivation and cultivation + sowing of a grass/legume mixture significantly reduced the number of *L. draba* plants at both sites, however only at the beginning of the field season (Iasi: $F_{2,10}$ = 18.23; Perieni: $F_{2,10}$ = 13.92; $P$ < 0.001; Fig. 1). On non-cultivated plots, plant numbers declined by over 50% between May and June, which was presumably due to interspecific competition (Iasi: $F_{2,47}$ = 103.88; Perieni: $F_{2,47}$ = 23.42; $P$ < 0.001). The exclusion of grazing in all blocks in 2006 considerably reduced disturbance in uncultivated plots and, in addition, allowed the associated vegetation to compete more effectively with *L. draba*.

Neither the number of shoots nor shoot height was significantly affected by any of the treatments. Insect attack on leaves was much higher in Iasi than in Perieni (Fig. 2), and pesticide applications only reduced leaf attack in Perieni ($F_{2,47}$ = 5.94; $P$ = 0.019). Overall, attack of leaves was very high, probably because pesticides were applied too late and in a too-low dosage. Attack of shoots and root crowns, which were visible from the outside, was low at both sites (Fig. 2). However, attack by *C. assimilis* and *P. wrasei* were likely underestimated because plants were only visually inspected for attack. In Iasi, sowing of a grass/legume mixture increased percent cover of legumes in July (29% vs. 39%; $F_{2,71}$ = 3.79; $P$ = 0.027). In both Iasi and Perieni, percent cover of grasses was highest in uncultivated plots, 25% and 56%, respectively (Iasi: $F_{2,71}$ = 3.47; $P$ = 0.037; Perieni: $F_{2,71}$ = 3.85; $P$ = 0.026), and cover of bare ground was lowest (Iasi: $F_{2,71}$ = 22.85, Perieni: $F_{2,71}$ = 23.32; $P$ < 0.001).

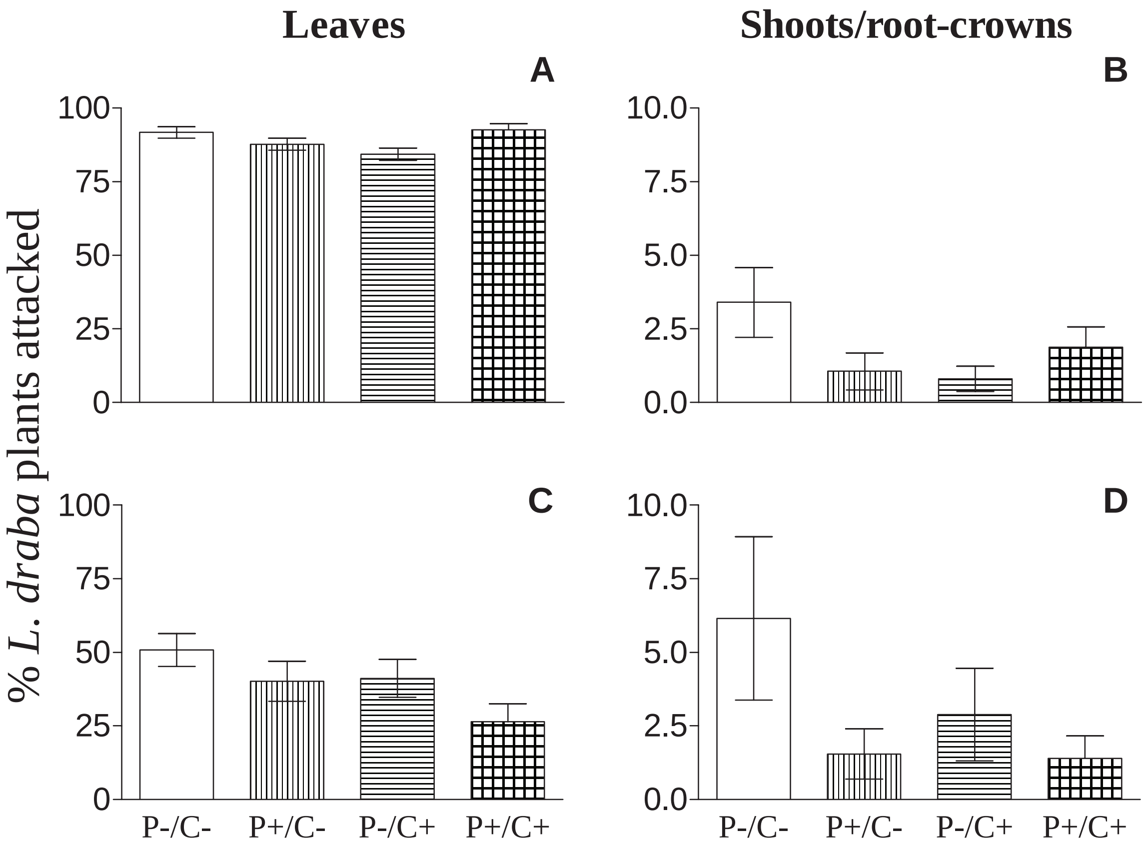


**Figure 2.** Effect of regular application of pesticides (P +/–) and carbon in the form of sawdust (C +/–) on the mean proportion (±SE) of *Lepidium draba* plants attacked in May 2006 at Iasi (a and b) and Perieni (c and d). Attack is based on visual examination of plants.

## Conclusions and outlook

As expected, neither pesticide nor sawdust applications had a large effect in the first year. To increase the effectiveness of pesticide applications, dosages are increased and treatments are started earlier in 2007. To obtain a better estimate of endophagous herbivory, we are also planning to dig up plants from the periphery of plots and dissect them. This will also allow us to correlate attack with individual plant traits. Cultivation treatments only had a temporary effect on *L. draba*.

In a review of studies on the impact of herbivores on plant population dynamics, Crawley (1989) found that vertebrate herbivory generally had a stronger impact on plant population dynamics than invertebrate herbivores. Only 6 weeks after fencing the blocks, we observed large differences between the areas inside and outside ungulate exclosures, i.e. vegetation outside ungulate exclosures was heavily overgrazed. We therefore established eight additional 3 × 3 m plots outside of exclosures in July 2006 as comparison.

In conclusion, we expect that grazing treatments will have a larger effect on *L. draba* growth and densities than phytophagous arthropods and that the effect of the latter and carbon addition will need more time to become apparent. The project will continue until Autumn 2008, when final conclusions will be drawn.

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# Assessing herbivore impact on a highly plastic annual vine

**J.A. Hough-Goldstein**[1]

*Summary*

Effective biological control of a weed population requires an understanding of the impact of herbivory on the target host plant. This can be complicated by phenotypic plasticity in response to environmental heterogeneity. *Polygonum perfoliatum* L. [also known as *Persicaria perfoliata* (L.) H. Gross], an invasive annual vine accidentally imported from Asia to the Mid-Atlantic region of the USA in the 1930s, has been the target of a biological control program since 1996. In 2004, a Chinese weevil, *Rhinoncomimus latipes* Korotyaev, was approved for release in North America. Cage studies in 2005 showed that *P. perfoliatum* is highly plastic in its response to light: Individual plants grown in cages produced over 2000 seeds per plant in full sun but fewer than 400 in the shade. In 2006, with all plants in full sun, weevils that were introduced into cages early in the season suppressed seed production for about 9 weeks, but plants were able to produce substantial numbers of seeds late in the year. Further studies with plants under conditions more closely approximating the field situation (e.g. with competition from other plants) are likely to show a greater impact of weevil herbivory on the plants.

***Keywords***: *Polygonum perfoliatum*, *Persicaria perfoliata*, *Rhinoncomimus latipes.*

## Introduction

Mile-a-minute weed (MAM), *Polygonum perfoliatum* L. [also known as *Persicaria perfoliata* (L.) H. Gross] is an alien invasive weed from Asia that infests natural areas in a variety of habitats in its imported range. This annual vine is a prolific seed producer and has become a serious problem in the Mid-Atlantic region of the USA. The North American population is thought to have originated near York, PA in the 1930s, probably introduced as a seed contaminant with holly seed imported from Japan (Moul, 1948). Although it was recognized as a potentially dangerous weed that should be eradicated, no action was taken, and the weed can now be found from Delaware west to Ohio, south to West Virginia and north to Massachusetts. A biological control program was initiated by the US Forest Service in 1996. Over 100 insect species were identified on MAM in China, including several that appeared to have a narrow host range (Ding *et al*., 2004). One of these, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), was tested on plant species in China and in quarantine in Delaware and found to be extremely host-specific (Price *et al*., 2003; Colpetzer *et al*., 2004). This insect was approved for release in the USA by the US Department of Agriculture's Animal and Plant Health Inspection Service in 2004.

Eggs of *R. latipes* are laid on MAM plants and hatch in about 5 days. Neonates crawl along stems and enter a node, where they feed internally for 1 to 2 weeks, after which they drop out of the stem and pupate in the soil. Adults emerge about 1 week later and feed on MAM leaves and terminals. The weevils go through multiple, overlapping generations until early to mid-September, when egg laying ceases (unpublished data). Adult weevils overwinter in the soil or leaf litter.

The weevils have been reared at the New Jersey Department of Agriculture Phillip Alampi Beneficial Insect Laboratory in Trenton, NJ, since autumn 2004, and in 2006, more than 20,000 weevils were reared and released. Most releases occurred in New Jersey, but insects have also been released and have established at sites in Delaware, Pennsylvania, West Virginia and Maryland. Although it is too soon to assess their impact in the field, plant mortality has been observed in some areas where weevils have heavily defoliated MAM plants in New Jersey.

To gain a better understanding of the potential impact of the weevil on *P. perfoliatum*, experiments were conducted in 2005 and 2006 using isolated MAM plants enclosed in weevil-proof cages with various levels of

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weevils applied at different times. The 2005 experiment also addressed the question of whether seed-cluster consumption by birds or deer may have a significant impact on *P. perfoliatum* seed production.

## Methods and materials

### 2005 Experiment

Controls consisted of single MAM plants completely enclosed in cages approximately 2 m tall and 0.9 m square, while treatments with weevils were the same but with 20 weevils per cage added on 21 July. Cages open to birds were identical except with open tops, and cages available to deer browse were ~1 m tall with open tops. No *R. latipes* were present in this area in 2005, and therefore, open cages were not exposed to weevils. All cages were made from white polyester netting material with a mesh of approximately 10 × 8 cm (BioQuip Products, Inc., Gardena, CA, USA), with a Velcro opening sewn into one corner and supported by frames constructed of 1.9 cm diameter polyvinyl chloride conduit pipe.

Five replicates were set up for each of the four treatments, assigned at random to 20 plants, approximately 4 m apart, growing naturally at a site in White Clay Creek State Park near Newark, DE. Other plants within ~ 0.5 m of the plants were removed on 6 June and again on 21 July when cages were installed. Each plant was provided with a tomato support extended with three bamboo poles wrapped in wire to support growth of the vine. The plants were about 1 m tall when treatments were applied. Nylon window screening was placed at the bottom of each cage to collect seeds as they fell from the plants.

The number of seed clusters per plant was recorded, and fallen seeds (achenes) were collected from the screening each week and counted. Relative plant size and relative light exposure (full sun, partial sun or shade) were also noted weekly. On 29 November, after all plants were dead, the remaining plant material was collected into large paper bags, left to dry in a greenhouse for 2 weeks and then weighed.

### 2006 Experiment

Thirty cages identical to the tall closed cages used in 2005 were placed over isolated *P. perfoliatum* plants at a different site in White Clay Creek State Park, approximately 1000 m away from the 2005 site, on 19 May 2006, when plants were about 30 cm tall. Cages were at least 4 m apart and were arrayed along the edge of a meadow in a randomized complete block design, so that plants in the same block had similar exposure to sun and most plants were exposed to full sun for much of the day. There were six replicate cages each of five treatments: early high, 20 weevils per cage added on 26 May; early low, five weevils per cage added on 26 May; late high, 20 weevils per cage added on 23 June; late low, five weevils per cage added on 23 June and control, no weevils added. Weevils were obtained from the Phillip Alampi Beneficial Insect Laboratory, Trenton, N.J. Although they were not sexed, they were assigned randomly to the different treatments, and spot checks at

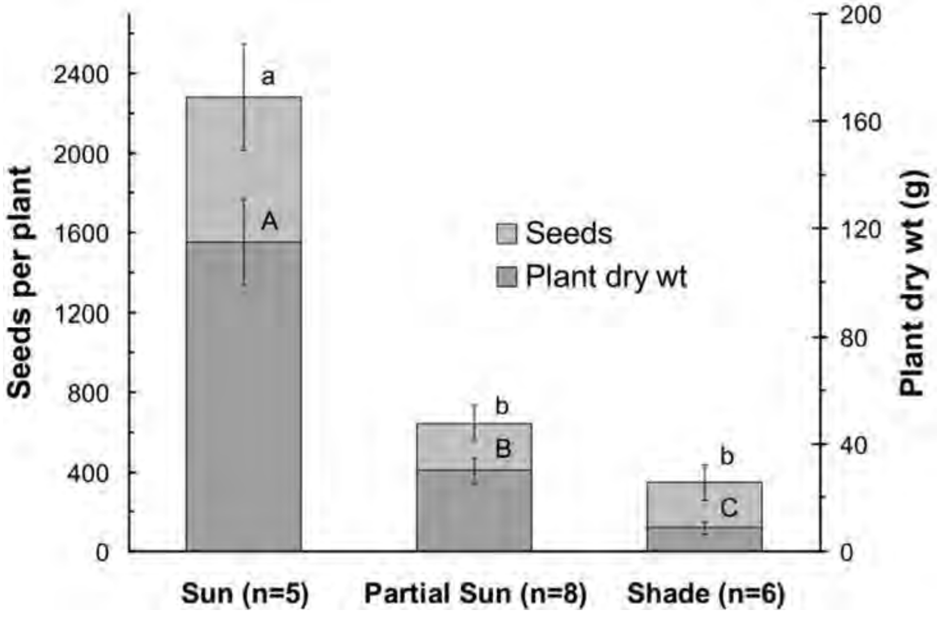


**Figure 1.** Effect of sun exposure on total numbers of seeds produced and plant dry weights (means ± SE) for single *Polygonum perfoliatum* plants enclosed in cages in 2005. Means with the *same lowercase* or *uppercase letter* are not significantly different (Tukey's test on square-root transformed data; untransformed means and standard errors are shown).

the New Jersey laboratory indicated a 1:1 sex ratio in reared weevils (D. Palmer, personal communication).

Cages were checked weekly for presence of defoliation, number of weevils that could be observed, presence of node damage and weevil eggs and number of seed clusters per plant. Seeds were collected from the screen at the base of the cages each week beginning in late July and counted in the laboratory. Plants were cut off at the base on 15 November and left to dry in paper bags for several weeks as in 2005, after which they were weighed.

## Statistical analyses

Data were transformed by square root ($x + 0.5$) to reduced heteroskedasticity of variance residuals. Transformed data were analyzed using two-way analysis of variance, by treatment and sun exposure in 2005 and by treatment and block in 2006. Tukey's test was used for mean separation. Non-transformed means and standard errors are presented in figures.

# Results

## 2005 Experiment

The total number of seeds produced by individual plants varied from a low of 39 for a small 'control' plant (enclosed in a closed cage, without weevils) growing in the shade, to a maximum of 3172 for a 'bird-exposed' plant (i.e. in a tall cage with an open top) growing in the full sun. There were no significant differences by

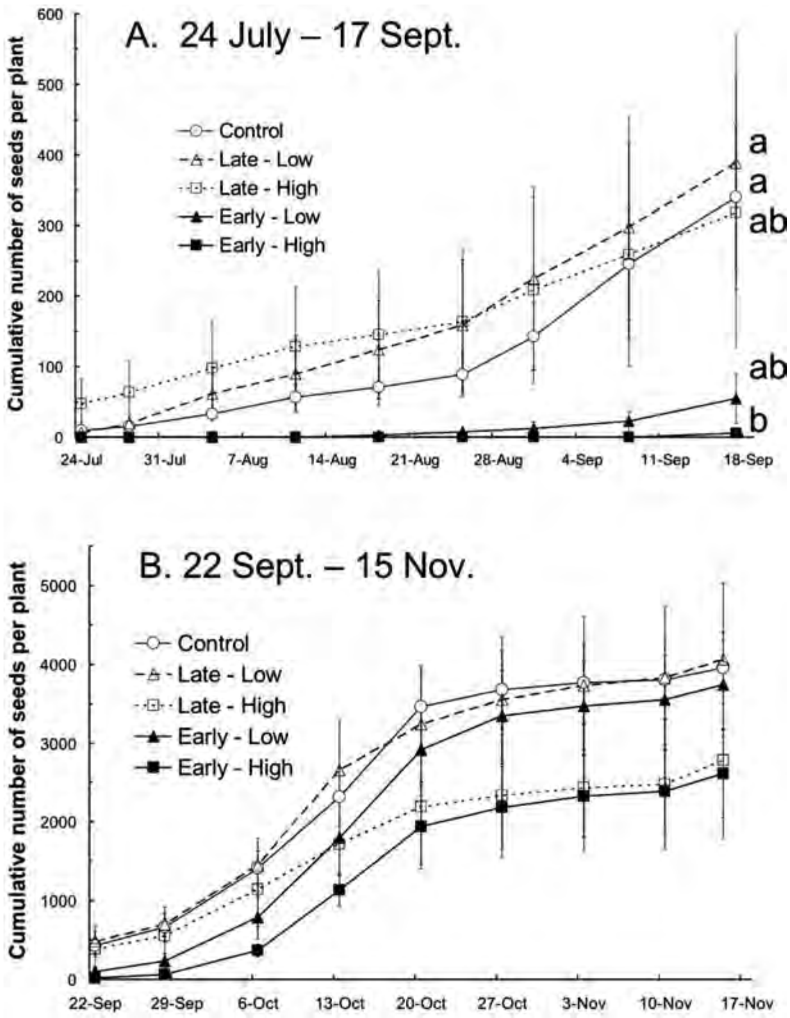


**Figure 2.** Effect of weevil treatments on cumulative total number of seeds produced (means ± SE) during **A** the first 9 weeks, and **B** the last 9 weeks of seed collection, for single *Polygonum perfoliatum* plants enclosed in cages in 2006. Means with the *same letter* are not significantly different (Tukey's test on square-root transformed data; untransformed means and standard errors are shown). 'Late' treatments had weevils added on 23 June, and 'Early' weevils were added on 26 May; 'Low' treatments received five weevils per cage and 'High' treatments received 20 weevils per cage.

cage treatment for total number of seeds ($F_{3,2}$ = 2.90, $P$ = 0.0754), plant dry weights ($F_{3,2}$ = 1.11, $P$ = 0.3809) or maximum number of seed clusters counted on plants (on 29 September: $F_{3,2}$ = 2.74, $P$ = 0.0858). However, differences in all of these parameters were highly significant by sun exposure (seed production, $F_{3,2}$ = 41.10, $P$ < 0.0001; plant dry weights, $F_{3,2}$ = 46.92, $P$ < 0.0001 and seed clusters, $F_{3,2}$ = 11.41, $P$ = 0.0014). Plants grown in full sun averaged over 2200 seeds per plant, while those in full or partial shade produced an average of 300–600 seeds per plant (Fig. 1). Plant dry weight differences were even more dramatic and differed among all three levels of sun exposure (Fig. 1).

### 2006 Experiment

Total seed production did not differ by treatment in 2006 ($F_{4,5}$ = 1.03, $P$ = 0.4175). However, there was a significant difference in the cumulative total number of seeds produced during the first 9 weeks that seeds were collected (24 July to 17 September: $F_{4,5}$ = 3.77, $P$ = 0.0213), with significantly more seeds produced on the control plants and the late low plants than on the early high plants (Fig. 2A). Differences by treatment were not significant for total numbers of seeds produced during the last 9 weeks ($F_{4,5}$ = 1.08, $P$ = 0.3938; Fig. 2B) or plant dry weights ($F_{4,5}$ = 0.18, $P$ = 0.9455). All treatments in 2006 averaged more than 2600 seeds per plant.

## Discussion

The 2005 study revealed the extreme plasticity of *P. perfoliatum* under different conditions of light exposure. Plants grown in full sun were more than ten times larger and produced more than six times as many seeds as plants grown in shade. Similar results were obtained by Sultan and Bazzaz (1993), who found very large differences in fruit and plant biomass produced by *Polygonum persicaria* L. under different light regimes in the greenhouse. These differences apparently swamped any that may have occurred due to minor feeding on seed clusters by deer or birds in the open cages or by weevils added to the cages in July in 2005.

In 2006, seed production was almost completely suppressed between late July and mid-September in plants with early application of weevils at the high level (20 weevils per plant). However, all plants produced numerous seeds in October, resulting in no significant difference by treatment in total seed production over the season. In this experiment, the caged plants were unusually large and robust, as most were growing in full sun and had minimal competition with other plants. Further studies on MAM plants under conditions more closely approximating the field situation, especially with competition from other plants, are likely to show a greater impact of weevil herbivory on the plants. Based on observations in the field, where large weevil populations have developed and plants are subject to normal competitive stress, complete seed suppression and plant mortality can occur.

As with many invasive plant species, MAM plants showed an impressive ability to recover from severe insect damage. Nevertheless, under conditions where weevil populations grow exponentially over several years, the insect may be able to act as an effective biological control agent.

## Acknowledgements

Megan Schiff, Ellen Lake, Brian Butterworth, Jamie Pool, Jason Graham, Matt Frye and Louisa Harding all contributed greatly to this project. Daniel Palmer and Amy Diercks, New Jersey Department of Agriculture, developed rearing methods and shared their knowledge and weevils. I also thank Richard Reardon for support through the Forest Health Technology Enterprise Team, USDA Forest Service, Morgantown, WV.

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# The disintegration of the Scrophulariaceae and the biological control of *Buddleja davidii*

**M.K. Kay,[1] B. Gresham,[1] R.L. Hill[2] and X. Zhang[3]**

*Summary*

The woody shrub buddleia, *Buddleja davidii* Franchet, is an escalating weed problem for a number of resource managers in temperate regions. The plant's taxonomic isolation within the Buddlejaceae was seen as beneficial for its biological control in both Europe and New Zealand. However, the recent revision of the Scrophulariaceae has returned *Buddleja* L. to the Scrophulariaceae *sensu stricto*. Although this proved of little consequence to the New Zealand situation, it may well compromise European biocontrol considerations. Host-specificity tests concluded that the biocontrol agent, *Cleopus japonicus* Wingelmüller (Coleoptera, Curculionidae), was safe to release in New Zealand. This leaf-feeding weevil proved capable of utilising a few non-target plants within the same clade as *Buddleja* but exhibited increased mortality and development times. The recent release of the weevil in New Zealand offers an opportunity to safely assess the risk of this agent to European species belonging to the Scrophulariaceae.

***Keywords***: *Cleopus*, *Buddleja*, taxonomic revision, phylogeny.

## Introduction

There are approximately 90 species of *Buddleja* L. indigenous to the Americas, Asia and Africa (Leeuwenberg, 1979), and a number have become naturalized outside their native ranges (Holm *et al.*, 1979). Buddleia, *Buddleja davidii* Franchet, in particular, is an escalating problem for resource managers in temperate regions and has been identified as a target for classical biological control in New Zealand (Kay and Smale, 1990) and Europe (Sheppard *et al.*, 2006).

Buddleia is a large woody shrub of Asian origin that was introduced to the rest of the world as an ornamental species in the 1890s. It was considered naturalized in the UK in the 1930s and in New Zealand in the 1940s (Esler, 1988). It has many of the features that characterize successful weed species, and it is ranked in the top ten invasive plants of Britain (Crawley, 1987). It matures quickly, is capable of flowering in its first year of life and produces an extraordinary number of small seeds that are efficiently dispersed by wind. However, there is no significant soil seed bank. The seed germinates almost immediately, and the density and rapid early growth of buddleia seedlings suppresses other pioneer species (Smale, 1990).

As a naturalized species, buddleia is a shade-intolerant colonizer of urban wastelands, riparian margins and other disturbed sites, where it may displace indigenous species, alter nutrient dynamics and impede access (Smale, 1990; Bellingham *et al.*, 2005). In New Zealand, on sites prepared for exotic forest plantations, the rapid growth of buddleia causes the suppression and a quantifiable loss of growth in newly planted *Pinus radiata* Don. (Richardson *et al.*, 1996). The inefficiencies of conventional controls prompted the investigation of classical biological control (Kay and Smale, 1990).

The taxonomic isolation of a target weed from indigenous and other valued non-target plant species reduces the risk posed by introduced biological control agents. However, taxonomy is far from an exact science, and the taxonomy of the paraphyletic *Buddleja* has had a chequered history. *Buddleja* has variously been placed within the families, Scrophulariaceae, Loganiaceae, the conveniently promoted Buddlejaceae and, most recently, returned to the Scrophulariaceae, which has been a recognized repository for undefined Lamiales (Tank *et al.*, 2006). The on-going reconstructing of the Scrophulariaceae combines morphological,

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embryological, molecular and chemical parameters, as well as the host preferences of specialist invertebrates (Stevens, 2001).

Fortunately, there are few close relatives of buddleia within the New Zealand indigenous Scrophulariaceae s.s., although the indigenous shrub, *Myoporum laetum* G. Forst., Myoporaceae, has now been relegated to the tribe Myoporeae, within the same clade as Buddlejeae (Tank *et al.*, 2006). Most other New Zealand genera previously placed in the Scrophulariaceae, including the manifold *Hebe* Comm. ex Juss., are now better defined in other clades within the Scrophulariaceae s*ensu lato*.

During a survey of insects and pathogens associated with *B. davidii* in China, *Cleopus japonicus* Wingelmüller (Coleoptera, Curculionidae) appeared to be a potential biological control agent because of its apparent host specificity and ubiquity. The adults and larvae feed externally on leaves. Eggs are oviposited singly within excavated leaf cavities, and the emergent slug-like larvae remain attached to the plant by secreting a coating of viscous fluid.

The host-specificity studies reported in this paper evaluated whether *C. japonicus* is a safe biological control agent for buddleia in New Zealand.

## Methods and materials

Preliminary trials conducted in China tested species from 16 plant families. *C. japonicus* was then imported into quarantine in New Zealand. The 76 plant taxa tested in New Zealand were selected following the internationally accepted 'centrifugal phylogenetic system' of Wapshere (1974). The relative susceptibility of 14 *Buddleja* taxa was tested. Thirty-five New Zealand indigenous plant species were tested, including *Geniostoma rupestre* J.R. Forst.& G. Forst., the only endemic representative of the Loganiaceae, and 21 species from the Scrophulariaceae s.l. Given the uncertain nature of *Buddleja* taxonomy, it was considered prudent to give extensive coverage of New Zealand scrophularia species, particularly the many species of *Hebe*. A further 11 species of Scrophulariaceae s.l. that are exotic to New Zealand were tested, along with 16 exotic species from other families that commonly grow in association with buddleia in New Zealand. The New Zealand trials were conducted in a quarantine insectary maintained at 20°C ± 2 and 70% ±10 RH, 14-h photoperiod.

Tests were run with naïve and pre-fed adults, in both choice and no-choice trials. The degree of feeding, oviposition and mortality was scored against that of insects placed on concurrent buddleia controls. No-choice larval trials utilized both pre-fed and naïve first instar larvae. To obtain naïve larvae, eggs of known age were monitored closely for larval eclosion. Emerging larvae were transferred to the test plant material before feeding.

## Results

A full account of trial results is available on the Environmental Risk Management Authority website (www.ermanz.org). Adult *C. japonicus* did not oviposit, or feed, on any of the 35 species belonging to 24 plant families outside of the Scrophulariaceae in either of the preliminary trials in China or the trials conducted in New Zealand. However, the weevil did lay a very small number of eggs on a few of the 21 New Zealand indigenous species within the family Scrophulariaceae s.l. These eggs were laid externally, rather than in purposefully excavated sites, and failed to produce larvae. Larvae transferred to these plants also developed poorly. Within the genus *Buddleja*, *C. japonicus* could complete development on all, except *Buddleja salviifolia* (L.) Lam and *Buddleja auriculata* Benth. but performed best and had a significant preference for *B. davidii* (Table 1).

Newly emerged larvae transferred to the foliage of 17 New Zealand indigenous *Hebe* species died quickly without completing development. One anomaly occurred when one larva of one replicate completed development to adult on the foliage of an ornamental specimen of *Hebe speciosa* (A.Cunn.) Ckn. & Allan. One larva also completed development on each of the indigenous *Limosella lineata* Glück, [Limosellae

**Table 1.** Summary of feeding and oviposition trials for *Cleopus japonicus* presented as a ranking of host suitability within the genus *Buddleja*. Ranking was determined from the combined rankings of larval and adult feeding and oviposition for each species.

| *Buddleja* species | Section | Origin | Rank |
|---|---|---|---|
| *B. davidii* Franch. | Neemda | SE Asia | 1 |
| var. *lochinch* | – | – | 2 |
| var. *weyeriana* | – | – | 3 |
| *B. madagascariensis* Lam. | Nicodemia | Madagascar | 3 |
| *B. japonica* Hemsl. | Neemda | SE Asia | 5 |
| *B. alterniflora* Maxim. | Neemda | SE Asia | 6 |
| *B. globosa* Hope | Neemda | S America | 7 |
| *B. lindleyana* Fortune | Neemda | SE Asia | 8 |
| *B. parviflora* H. B. K. | Neemda | N America | 9 |
| *B. asiatica* Lour. | Neemda | SE Asia | 10 |
| *B. colvillei* Hook. f. et Thoms | Neemda | India | 10 |
| *B. dysophylla* (Benth.) Radlk. | Chilianthus | S Africa | 12 |
| *B. auriculata* Benth. | Neemda | S Africa | 13 |
| *B. salviifolia* (L.) Lam. | Neemda | S Africa | 14 |

(Scroph I.)] and *Glossostigma elatinoides* Benth. ex Hook. f. [Phrymaceae (Scroph IV)], but adult weevils did not oviposit on these species.

Most exotic scrophularia appeared to be immune to attack by the weevils, but larval and adult feeding and oviposition occurred on the weedy European species *Verbascum thapsus* L., *Verbascum virgatum* Stokes and *Scrophularia auriculata* L.

## Discussion

*Cleopus* Dejean, belonging to a tribe (Cionini) of host-specific 'figwort' weevils and the European representatives (*Cionus* Clairville and *Cleopus* species), feed on *Scrophularia*, *Verbascum* and occasionally on adventive *Buddleja* (Walker, 1914, Hoffman, 1958; Cunningham, 1974, 1975; Williams, 1974; Read, 1976, 1978; Bullock, 1987; Smith, 1992). Conversely, the Asian species, *C. japonicus*, has only been recorded from *B. davidii* (Zhang *et al.*, 1993), and this study found that it could only complete its life cycle on a few *Buddleja* taxa, but could feed on *Scrophularia* and *Verbascum*. The host associations of these species appear to support the recent revision of the Scrophulariaceae (Fig. 1).

Other invertebrates are also known to feed exclusively on these plant species, which have been recognized as a distinct clade, Scrophulariaceae s.s. ['Scroph I' of Olmstead and Reeves (1995) and Olmstead *et al.*, (2001)] within the Scrophulariaceae s.l. Allen (1960) noted that weevils of the Gymnetrini distinguished between the Plantaginaceae (Scroph II) and the Scroph I clade and that they fed indifferently upon *Scrophularia* and *Verbascum*.

Westwood (1849 in Scott 1937) remarked that *Cionus scrophulariae* L.;

"…long ago discovered the Natural System, and proved by the fact of their sometimes indiscriminately feeding on mulleins [*Verbascum*] and figworts [*Scrophularia*] that these plants were in truth closely allied in Nature."

Scott (1937) records the same weevil feeding on the introduced Cape figwort *Phygelius capensis* Benth. (Scroph I) and notes the observations, by others, of *Cionus* and *Cleopus* occasionally feeding on introduced *Buddleja* in the UK. In the UK, the only specialist Lepidopteran to occasionally feed on buddleia is the mullein moth, *Cucullia verbasci* L., which normally has the same host range as the figwort weevils (Owen and Whiteway, 1980). The flea beetles, *Longitarsus* spp. Latreille, also only have the hosts *Scrophularia* and *Verbascum*, and in summary of these observations, Allen (1960) stated: "Yet none of these insects, apparently, is known ever to attack *Lanaria* or *Antirrhinum* [both of Antirrhineae, Scroph II ] in a state of nature, and I am aware of no instance of a *Linaria* feeder (of which there are many) having *Scrophularia* as a host."

Elements of the distinctive iridoid and terpenoid phytochemistry of *Buddleja* have been shown to be

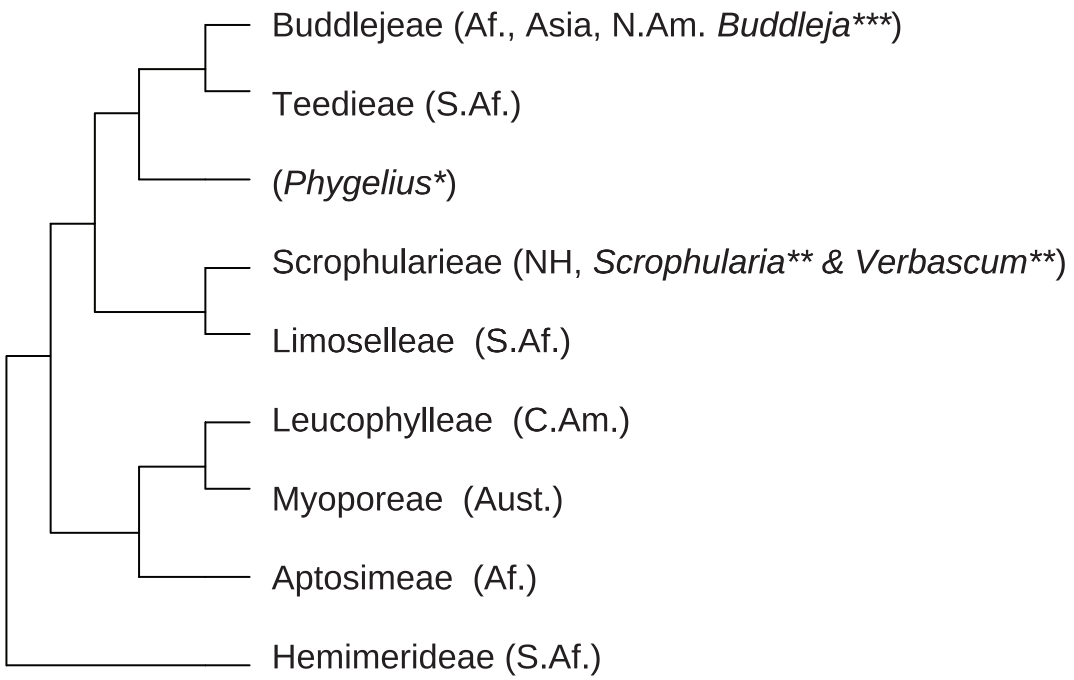


**Figure 1.** Summary of the phylogenetic relationships among the tribes and the unresolved genus, *Phygelius*, of the Scrophulariaceae *sensu stricto* (after Tank *et al.*, 2006). Low (*single asterisk*) to high level (*triple asterisk*) of feeding by *Cleopus japonicus*.

biologically active (Yoshida *et al.*, 1976; Houghton *et al.*, 2003) and may well influence the invertebrate feeding guild associated with this and closely related genera. Iridoids are known to be feeding stimulants for specialist Lepidoptera (Bowers, 1988) and deterrents for generalists (Stephenson, 1982).

The study reported in this paper not only confirms the restricted host preference of the Cionini and demonstrates the low risk of *C. japonicus* to the New Zealand flora but also supports the current position of *Buddleja* within the new taxonomy. Bearing in mind that laboratory studies are thought to overestimate the host range of potential biological control agents (Hill, 1999), the host range of *C. japonicus* in the field may be more limited. However, these results cannot preclude the possibility that, if released, *C. japonicus* could produce self-sustaining populations on *Verbascum*, *Scrophularia* and *Buddleja* species. Four other species of *Buddleja* [*B. salviifolia* L., *Buddleja madagascariensis* Lam., *Buddleja globosa* Hope and *B. dysophylla* (Benth.) Radlk.] have already partially naturalized in New Zealand. The early flowering *B. salviifolia* is valued as a spring nectar source for bees (Kay and Smale, 1990). However, *C. japonicus* adults fed poorly, larvae failed to feed and no eggs were laid on *B. salviifolia*. It is unlikely that this species would be colonized by *C. japonicus*. In contrast, *B. madagascariensis* is one of a number of *Buddleja* species to be considered strongly invasive on the west coast of USA and Hawaii (Randall and Marinelli, 1996). It ranked highly as a host of *C. japonicus*. *B. globosa* is not considered to be invasive, and any attack by *C. japonicus* may limit the potential ornamental value of this species. *B. dysophylla* appeared to be a poor host for *C. japonicus*, but it is rarely cultivated in New Zealand and is not considered invasive.

*C. japonicus* appears to have a restricted host range and could be expected to be at least as host-specific as its European congeners. We conclude that it is possible that *C. japonicus* may cause minor damage to some non-target scrophulareous species, particularly within *Buddleja*, *Verbascum* and *Scrophularia*. These are all weedy species or ornamentals of minor importance in New Zealand, but in Europe, the tribe Scrophularieae exhibits considerable radiation, resulting in 400–500 species of *Scrophularia* and *Verbascum*. A number of these are rare or endangered (Wigginton, 1999), and rigorous testing would be advisable.

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# Quarantine evaluation of *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), a potential biological control agent of tree of heaven, *Ailanthus altissima*, in Virginia, USA

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*Summary*

Tree of heaven, *Ailanthus altissima* (Mill.) Swingle, is an imported invasive weed tree from China that has become established throughout much of the continental USA. It colonizes disturbed forest sites and often out-competes native vegetation. Short-term cultural and chemical controls of this weed are expensive and have limited efficacy. *Eucryptorrhynchus brandti* (Harold) and *Eucryptorrhynchus chinensis* (Olivier), two curculionid species, are pests of *A. altissima* in China and have no other known hosts. The objectives of our project are to (1) assess the pest status of *A. altissima* in Virginia and (2) evaluate *E. brandti* for its potential as a biological control agent. A statewide survey showed significant presence of tree of heaven but no native herbivores with potential of controlling it, suggesting biological control to be an attractive method of management. As *E. brandti* requires live trees for development, quarantine studies have focused on developing a rearing technique and testing the host specificity on native plants approved by the Technical Advisory Group for Biological Control Agents of Weeds. Preliminary results indicate that *E. brandti* feeds only on tree of heaven, with greatly reduced feeding observed on corkwood, *Leitneria floridana* Chapman, and paradise tree, *Simarouba glauca* DC.

***Keywords***: rearing, *Eucryptorrhynchus brandti*, host specificity testing, natural enemy.

## Introduction

*Ailanthus altissima*, tree of heaven, is an introduced species in Europe (Ballero *et al.*, 2003; Lenzin *et al.*, 2004), Africa, South America and North America (Ding *et al.*, 2006). Seeds were introduced from China to Paris between 1740 and 1750 (Hu, 1979; Tellman, 2002) and in North America as an ornamental shade tree during the late 18th century from Europe into Philadelphia, Pennsylvania (Feret, 1985; Tellman, 1997). Multiple introductions into New York occurred during the early 19th century (Davies, 1942; Dame and Brooks, 1972; Hu, 1979).

Currently, tree of heaven is found in 41 of the lower 48 continental USA from Washington to New England and south to Florida, Texas and Southern California (USDA-NRCS Plants Database, 2007). It is often used as an ornamental adjacent to sidewalks, streets and in parking lots. In Virginia, tree of heaven is a dominant species along roadsides and occupies hundreds of acres in the Shenandoah National Park (Marler, 2000).

Tree of heaven has many beneficial attributes and often is regarded as an important ornamental species in the countries of origin because of its aesthetic value and ability to withstand environmental pollutants and water stress in an environment caused by human activities (Ding *et al.*, 2006). Traditional Chinese culture has used tree of heaven for its anti-tumour properties (Ammirante *et al.*, 2006). In its native range, tree of heaven is fed upon by more than 40 phytophagous arthropod species and is susceptible to nearly 20 pathogens (Ding

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*et al.*, 2006). Few pathogens occur in the USA, and only one has caused isolated fatality of an individual tree of heaven plant (Ding *et al.*, 2006). None of the pathogens occurring in the USA appears to be specific to tree of heaven (Ding *et al.*, 2006). Tree of heaven produces allelopathic compounds capable of inhibiting the growth of nearly 90 tree species (Mergen, 1959; Heisey, 1990a,b; Lawrence *et al.*, 1991; Heisey and Heisey, 2003). The lack of natural enemies of tree of heaven and its ability to suppress plant growth over a wide range of habitats allow it to out-compete native flora in North America.

Chemical control of tree of heaven is the most common control method. Herbicides registered for tree of heaven control are dicamba, glyphosate, imazapyr, metasulfuron methyl and triclopyr. They are applied as foliar sprays, basal-bark treatments, injection or applied to cut stumps. However, chemicals often cause treated sites to be barren of any plant life resulting in the re-intrusion of invasive species like tree of heaven. There is a concern that continued large-scale herbicide applications may become detrimental to the environment in addition to being labor intensive and costly. For example, at a heavily infested 4-ha site on the median of interstate 81 in Virginia, the Virginia Department of Transportation estimated that the cost to control tree of heaven was $8750 per ha and produced only 'reasonable' control.

The lack of natural enemies of tree of heaven in the USA and the potential for biological control led to foreign exploration to identify potential biocontrol agents in China. A survey of the Chinese literature suggested that *Eucryptorrhynchus brandti* Harold and *Eucryptorrhynchus chinensis* (Olivier) (Coleoptera: Curculionidae) would be potential agents to investigate (Ding *et al.*, 2006). *E. brandti* is a univoltine beetle species native to China where it is considered a pest. In some areas of China, 80% to 100% of tree of heaven trees surveyed were attacked by *E. brandti* and *E. chinensis* causing 12% to 37% mortality (Ge, 2000; Ding *et al.*, 2006). Chinese people make considerable efforts to control *E. brandti* with chemicals and have found a nematode (*Steinernema feltiae*) that can produce 70% mortality in the field (Dong *et al.*, 1993; Jianguang *et al.*, 2004). The general biology of *E. brandti* is not well known. However, its development is probably similar to that of other curculionid species associated with woody trees (Barrett, 1967). Adults feed on leaves, buds and petioles (Ding *et al.*, 2006). Larvae develop under the bark and emerge as adults, leaving round emergence holes approximately 4 mm in diameter.

Our goal was to identify insect herbivores that can reduce the spread of tree of heaven. Specific objectives were to (1) survey for native insect herbivory on *A. altissima* in different regions of Virginia to identify any species with potential to impact tree of heaven and (2) import selected herbivores from the native habitat of *A. altissima* for host-specificity evaluation under quarantine to determine their potential for survival and development in Virginia.

# Materials and methods

## Tree of heaven in Virginia

Locations distributed throughout three different regions of Virginia were identified to determine the extent of *A. altissima* colonization. The regions were Ridge and Valley (Appalachian Mountains), Piedmont and Coastal Plain (Fig. 1). Within each region, at least two sites each along highways, rights-of-way or natural forest disturbance areas were selected. At each site, all tree species and their percent cover within the tree of heaven infestation were recorded. Observations of herbivores feeding on tree-of-heaven were done by visually examining at least 200 leaflets for herbivores. Herbivores were also collected by beating leaves over 1 $m^2$ beat sheets. Any herbivores found were collected and identified. Insect sampling also consisted of whole tree observation for activity and damage from insects. Whole tree observation included detailed examination of foliage, stems, buds and seeds (when available). Where obvious insect damage was detected, insects were collected if located. Our objective was not to carry out a biodiversity study or complete census of insects found on tree of heaven but to focus on insects colonizing or feeding on tree tissue causing observable damage. The survey began in the summer of 2004 and continued in 2005. Each site was visited monthly during the growing season (May to October).

Concurrent with the survey effort was the assessment of impact of identified herbivores on the growth, reproduction and survival of tree of heaven. When damage associated with insect feeding or colonization was found, it was followed by more intensive sampling of the causal agents. The intention was to determine, for each identified herbivore, the level and timing of activity across all regions and site types. We recognize that native herbivores are not likely to effectively control tree of heaven, as the weed has been highly successful thus far. However, this information will be helpful, as we evaluate exotic biological control agents and their potential interaction with our existing fauna.

## Quarantine testing of an exotic weevil imported from China

As part of a collaborative project with Dr. Ding Jianqing, Biological Control Institute, Chinese Academy of Agricultural Sciences, two weevil species, *E. brandti* and *E. chinensis*, identified as important pests of *A. altissima*, were studied in China. *E. brandti* was imported into the US Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) approved Quarantine facility at Virginia Tech beginning in 2004.

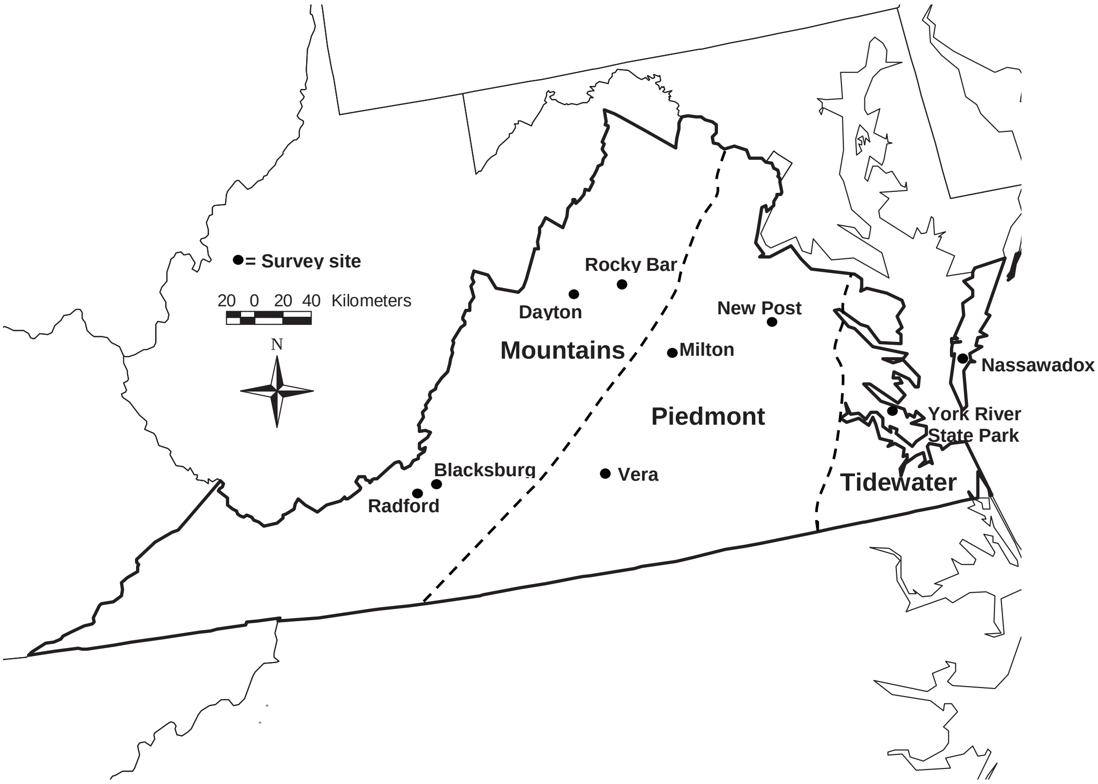


**Figure 1.** *Ailanthus altissima* (Mill.) Swingle survey sites in Virginia, USA: (1) Mountain, including Radford (forest), Blacksburg (forest), Dayton (forest) and Rockbar (roadside); (2) Piedmont, including Vera (forest), Milton (roadside) and New Post (roadside); (3) Coastal plain, including York River State Park (roadside) and Nassawadox (forest).

**Survival and development:** Adult *E. brandti* is reared on tree of heaven foliage and stems in containers at several constant temperatures (20°C to 30°C) within the range that the species will encounter in the US release areas. Biological data recorded included survival and development times of the egg, larval and adult stages, as well as fecundity of the females and egg hatch rates. Two colonies were established; one colony was maintained for production and the second used for biological and host specificity studies.

**Production colony:** This colony has been maintained and caged in screened plastic boxes (30 × 15 × 165 cm) at 22°C. Groups of five males and five females were placed in the cages. In each cage, tree of heaven billets, with the upper ends sealed with heated paraffin to reduce desiccation, were provided for oviposition and foliage added for adult feeding. Vermiculite was added to the bottom of the cage and kept moist. Weekly, billets were removed and placed in separate cages for larval development. Newly emerged adults were transferred to new oviposition cages.

**Fecundity:** Single pairs of male and female were caged in screened plastic boxes, as in the production colony cages, and replicated. Tree of heaven billets were added and checked for oviposition daily by removing the bark and examining the cambium for eggs. After initiation of oviposition, the bark was checked daily for eggs.

**Egg and larval development:** Eggs recovered from the billets were placed in Petri dishes with moistened filter paper and reared at several constant temperatures and checked daily for hatching. Newly hatched larvae were inoculated in tree of heaven billets by drilling a 7-mm diameter hole into the cambium and inserting one larva into the hole. Billets were checked at an appropriate time after inoculation to determine when development to the second instar occurred. This procedure was repeated at least three times for subsequent instars until pupation.

**Physiological host specificity testing:** Host-range studies included a series of no-choice and choice feeding and oviposition tests. Test plant species were from three groups, with a total of 30 species: 18 taxonomically related species (Simaroubaceae, Meliaceae and Rutaceae), six ecologically related species, and six economically related species. The taxonomically related species were chosen based on Wapshere's method of centrifugal phylogentic testing (Wapshere, 1974). This method involves exposing the biological control agent to a sequence of plants from those most closely related to the target species progressing to successively more and more distantly related plants until the host range

**Table 1.** Plant species to be tested for their suitability as hosts of *Eucryptorrhynchus brandti* (Harold). Species are listed with the most closely related listed first and the most distant last.

| Family | Species | Common name |
|---|---|---|
| Simaroubaceae | Chinese *Ailanthus altissima* (Mill.) Swingle | Tree of heaven |
| | *Simarouba glauca* DC | Paradise tree |
| | *Simarouba tulae* Urban | Aceitillo falso |
| | *Leitneria floridana* Chapman | Corkwood |
| | *Castela emoryi* (Gray) Moran & Felger | Crucifixion thorn |
| | *Castela erecta* Turp. | Cockspur, goat-bush, retama, rupagüita |
| | *Holacantha stewartii* C. H. Muell. | Stewart crucifixion thorn |
| Picramniaceae | *Alvaradoa amorphoides* Liebm. | Mexican alvaradoa |
| | *Picramnia pentandra* Sw. | Florida bitterbush |
| Meliaceae | *Swietenia mahagoni* (L.) Jacq. | West Indian mahogany |
| | *Citrus aurantifolia* (Christm.) Swingle | Lime |
| | *Citrus aurantium* L. | Sour orange |
| | *Citrus limon* (L.) Burm. F. | Lemon |
| | *Citrus paradisi* Macfad. | Grapefruit |
| | *Citrus reticulate* Blanco | Tangerine |
| | *Citrus sinensis* Osbeck | Sweet orange |
| | *Ptelea trifoliate* L. | Common hop tree |
| | *Zanthoxylum americanum* Mill. | Northern prickly-ash |

is thoroughly determined. The ecologically associated species were chosen based on our observations in Virginia. This species list is currently under review by the Technical Advisory Group (TAG) USDA/APHIS. A suitable host is defined as a plant species that will support feeding, oviposition and larval development of the insect to the adult stage, and the latter is capable of producing viable progeny. If any of these four conditions is not met, the insect will not be able to survive and is therefore not able to sustain a population solely on the plant species in question.

**Table 2.** Economically important and ecologically associated plant species to be tested.

| Family | Species | Common name |
|---|---|---|
| **Economically important** | | |
| Aceraceae | *Acer rubrum* L. | Red maple |
| Fagaceae | *Quercus alba* L. | White oak |
| | *Quercus rubra* L. | Red oak |
| Juglandaceae | *Carya glabra* (Mill.) Sweet | Pignut hickory |
| | *Juglans nigra* L. | Black walnut |
| Magnoliaceae | *Liriodendron tulipifera* L. | Tulip poplar |
| **Ecologically associated** | | |
| Anacardiaceae | *Rhus typhina* L. | Staghorn sumac |
| Cuppressaceae | *Juniperus virginiana* L. | Eastern redcedar |
| Leguminosae | *Robinia pseudoacacia* L. | Black locust |
| Pinaceae | *Pinus virginiana* Mill. | Virginia pine |
| Rosaceae | *Crataegus* spp. | Hawthorne |
| | *Prunus serotina* Ehrh. | Black cherry |

**Feeding tests:** Foliage and billets of the species listed in Tables 1 and 2 were used in choice and no-choice tests to date. Tests were conducted for 7 days at 20°C.

**No-choice.** Leaf clusters of each test plant species were caged with three adults. The amount of feeding was determined by placing a transparent millimeter square grid over the fed upon portion of the leaves. The control cage maintained at the same time with tree of heaven was without weevils.

**Choice.** Two series of tests were conducted, one with tree of heaven in the cage and one without. In the first test series, one cluster of tree of heaven leaves together with leaf clusters of one or more non-target species was placed in cages with adult beetles. In the second series, two or more non-target test species based on availability were placed in the cage with no tree of heaven. The available target species were selected randomly. Feeding was recorded as described above.

**Oviposition tests: No-choice.** Billets of one test plant species were placed in cages with one gravid female and compared with control billets of tree of heaven without weevils. The cambium was examined for eggs after 1 week. Foliage of the same species as the billet was placed in the cage with the adults for food.

**Choice.** Two or more species of billets were placed in cages with one gravid female. Two series of tests were carried out, with tree of heaven plus one or more other species in the cage and one with only a non-target

**Table 3.** Tree species composition and their percent coverage (%) at nine survey sites in Virginia, USA.

| Species | Mountain | | | | Piedmont | | | Coastal plain | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dayton | RockBar | Blacksburg | Radford | New Post | Milton | Vera | Nassawadox | York River State Park | |
| *Ailanthus altissima* (P. Mill.) Swingle | 15 | 85 | 80 | 30 | 35 | 70 | 15 | 20 | 15 | 32 |
| *Quercus palustris* Muenchh | 25 | 5 | | | 15 | | 60 | 10 | 15 | 14 |
| *Pinus virginiana* P. Mill. | | | | 95 | | | | | | 11 |
| *Juniperus virginiana* L. | | 10 | | | 15 | 10 | 15 | | | 6 |
| *Pinus taeda* L. | | | | | | | | 40 | | 4 |
| *Robinia pseudoacacia* L. | 15 | 10 | 15 | | | | 20 | | | 5 |
| *Liquidambar styraciflua* L. | | | | | 10 | | | 15 | 40 | 7 |
| *Pinus strobi* L. | 10 | | | | | 50 | | | | 7 |
| *Rhus glabra* L. | 20 | 15 | | | | | | | | 4 |
| *Prunus virginiana* L. | 15 | 10 | 25 | 20 | 5 | | | | | 6 |
| *Liriodendron tulipifera* L. | 20 | | | | 20 | | | | | 4 |
| *Acer negundo* L. | | 10 | | | | 15 | | | | 3 |
| *Ilex opaca* Ait. | | | | | | | | 30 | | 3 |
| *Juglans nigra* L. | | | | | 5 | | | | 20 | 3 |
| *Acer rubrum* L. | 10 | 10 | | | | | | | | 2 |
| *Ulmus americana* L. | | | | | 5 | | | | | 1 |
| *Acer saccharinum* L. | | | | | | 10 | | | | 1 |

test plant species. Foliage was added for food. After 1 week, the billets were checked for eggs.

# Results

## Survey of VA and impact assessment of native herbivores

Seventeen tree species were found to co-exist with tree of heaven in Virginia, with coverage of tree of heaven ranging from 15% to 85% (Table 3). The common associates found with tree of heaven were *Quercus* spp., observed in six of the nine survey sites, with coverage ranging from 5% to 60%. Twenty insect herbivore species were collected from tree of heaven in 2004 and 2005 (Table 4). The 12 beetles (Coleoptera) in Table 4 had little impact on tree of heaven. Their potential as biological control agents is minimal because of their low abundance and broad host range. The only abundant Coleoptera herbivore that may be causing serious damage to tree of heaven are the ambrosia beetles *Euwallacea validus* (Eichoff) and *Xyleborus atratus* Eichoff. These emerged from dying tree of heaven. We suspect that these species only attacked the dying or dead trees and had little effect on healthy tree of heaven. Based on our observations of herbivores in Virginia in 2004 and 2005, these herbivores had a negligible impact on tree of heaven.

**Table 4.** Insect herbivores found on *Ailanthus altissima* (Mill.) Swingle in 2004 and 2005 from nine survey sites in Virginia, USA.

| Herbivores | Common Name | Number individuals/ site |
|---|---|---|
| **Coleoptera** | | |
| *Odontota dorsalis* (Thunberg) | Locust leaf miner | 0.7 |
| Chrysomelidae spp. | Leaf beetle | 2.5 |
| Bruchid spp. | Seed beetle | 1.3 |
| *Popillia japonica* Newman | Japanese beetle | 3.4 |
| *Neotrichophorus* spp. | Click beetle | 5.2 |
| *Chrysolina quadrigemina* Suffrian | Flea beetle | 0.3 |
| *Apion* spp. | Weevil | <0.1 |
| *Merhynchites* spp. | Leaf rolling weevil | <0.1 |
| *Sphenophorus* spp. | Snout beetle | 0.3 |
| *Orchestes* spp. | Weevil | <0.1 |
| Scolytinae spp. | Ambrosia beetle | <0.1 |
| **Lepidoptera** | | <0.1 |
| *Ectropis crepuscularia* D. and S. | The small engrailed | <0.1 |
| *Thyridopteryx ephemeraeformis* (Haworth) | Bagworm | <0.1 |
| *Atteva punctella* (Cram.) | *Ailanthus* webworm moth | >30 |
| Saturniidae spp. | Silkworm moths | <0.1 |
| **Hemiptera** | | |
| *Empoasca* sp. | Leaf hopper | 0.7 |
| *Anormenis* sp. | Plant hopper | 2.3 |
| *Acanalonia* sp. | Plant hopper | 1.2 |
| **Orthoptera** | | |
| *Scudderia furcata* Brunner | Katydid | 0.5 |

**Table 5.** Mean (±SD) number of webworm, *Atteva punctella* Cramer, including eggs, larvae, pupae and adults, in 2004 and 2005, at three survey regions: Piedmont, Mountain and Coastal Plain.

| Month | Region | | |
|---|---|---|---|
| | Mountain | Piedmont | Coastal Plain |
| **2004** | | | |
| June | 4.0 ± 1.4 | 37 | 28 |
| July | 11.5 ± 8.9 | 54.3 ± 17.9 | 37.5 ± 26.2 |
| Aug. | 46.5 ± 17.0 | 91.3 ± 15.0 | 58.0 ± 2.8 |
| Sept. | 36.3 ± 30.4 | 21.7 ± 13.0 | 5.0 ± 5.7 |
| **2005** | | | |
| May | 2.0 ± 2.8 | 0.3 ± 0.6 | 0 |
| June | 6.5 ± 7.5 | 17.0 ± 23.6 | 21.0 ± 5.7 |
| July | 12.0 ± 5.7 | 111.0 ± 163.7 | 75.0 ± 19.8 |
| Aug. | 12.5 ± 10.6 | 46.3 ± 55.6 | 84.5 ± 50.2 |
| Sept. | 102.5 ± 88.4 | 106.0 ± 112.2 | 52.5 ± 31.8 |

Ailanthus webworm, *Atteva punctella* Cramer, was the only herbivore consistently present in all sites with a total of over 30 (eggs, larvae, pupae and adults) per visit. *A. punctella* caused ≥50% defoliation for 1-year-old seedlings. However, its effect on larger trees (>3 cm diameter) was minimal, causing less than 5% defoliation with no visible impact. The population of this species peaked around August (Table 5) with no significant difference among the three geographic regions [$F_{(22,11)} = 0.63$, $p = 0.83$) Two other insect species have been reported to feed on tree of heaven foliage: Cynthia moth, *Samia cynthia* (Drury), and the Asiatic garden weevil, *Maladera castanea* (Arrow) in eastern USA (Drooz, 1985). However, their presence was not identified in this survey, and it is unlikely that these two insect species will have any impact on the tree of heaven in Virginia.

## Quarantine testing of *E. brandti* imported from China: Development and rearing of *E. brandti* in the laboratory

A total of 500 and 1200 *E. brandti* adults were received from our cooperator in China in 2005 and 2006, respectively. In 2005, we initiated a study to evaluate the optimum conditions for rearing *E. brandti*. Eighteen adults (nine females, seven males and two unsexed) emerged from tree of heaven billets in Spring

**Table 6.** No choice foliage feeding tests of *Eucryptorrhynchus brandti* (Harold) adults on target and test species.

| Family | Species | Common name | *N* | $\bar{X}$ ± SD (mm$^2$ per adult per day) |
|---|---|---|---|---|
| Simaroubaceae | *Ailanthus altissima* (Mill.) Swingle | Tree of heaven | 9 | 56.4 ± 21.0[a]a |
| | *Simarouba glauca* DC | Paradise tree | 9 | 8.5 ± 0.4.8 b |
| | *Leitneria floridana* Chapman | Corkwood | 9 | 21.0 ± 9.2 b |
| Rutaceae | *Citrus aurantifolia* (Christm.) Swingle | Lime | 3 | 0 c |
| | *Citrus aurantium* L. | Sour orange | 3 | 0 c |
| Aceraceae | *Acer rubrum* L. | Red maple | 4 | 0 c |
| Anacardiaceae | *Rhus typhina* L. | Staghorn sumac | 4 | 0 c |
| Cupressaceae | *Juniperus virginiana* L. | Eastern redcedar | 4 | 0 c |
| Fagaceae | *Quercus alba* L. | White oak | 2 | 0 c |
| | *Quercus ruba* L | Red oak | 2 | 0 c |
| Juglandaceae | *Carya glabra* (Mill.) Sweet | Pignut hickory | 4 | 0 c |
| | *Juglans nigra* L. | Black walnut | 2 | 0 c |
| Leguminosae | *Robinia pseudoacacia* L. | Black locust | 4 | 0 c |
| Magnoliaceae | *Liriodendron tulipifera* L. | Tulip poplar | 4 | 0 c |
| Pinaceae | *Pinus virginiana* Mill. | Virginia pine | 2 | 0 c |
| Rosaceae | *Crataegus* spp. | Hawthorne | 4 | 0 c |
| | *Prunus serotina* Ehrh. | Black cherry | 2 | 0 c |

[a] Means within a column followed by different letters are significantly different at $P < 0.05$, Tukey–Kramer multiple comparison test.

2006, indicating that the weevil could complete its life cycle in a cut tree of heaven log.

At approximately 20°C, most *E. brandti* developed from egg to adult in 3 months. However, some individuals did not complete within 9 months and were still larvae after 9 months.

*E. brandti* did not expel frass from the billet. Frass remained in the billet within the feeding galleries. This made it difficult to know where the weevils were located and their life stage without dissecting the billet. Only 15% (18/119) of the weevils completed their life cycle and emerged. A few weevils that completed development failed to emerge, possibly due to poor food quality and quantity. Three young larvae were removed from one billet and transferred to another by inserting them into a 7-mm diameter hole. Frass was observed 3, 6, 9 and 11 days after the transfer, and two larvae completed development to the adult stage. They developed into adults but died inside the tunnel. This suggests that transfer of larvae into an artificially drilled hole has the potential to be used as a bioassay to test larval development on non-target species.

Based on the above observations, we developed a rearing procedure in 2006. Live tree of heaven trees were periodically cut into 1-m lengths, with diameter ranging from 10 to 22 cm. One end was treated with paraffin and the other end was placed in a 5-cm water bath to help maintain viable phloem tissue as long as possible. Groups of four billets were placed in a cage together with up to 150 weevils (male and female) for

**Table 7.** Two choice foliage feeding tests of *Eucryptorrhynchus brandti* (Harold) adults on target and test plant species.

| Family | Species | Common name | *n* | Test species | *A. altissima* |
|---|---|---|---|---|---|
| | | | | $\bar{X}$ ± SD (mm$^2$ per adult per day) | $\bar{X}$ ± SD (mm$^2$ per adult per day) |
| Simaroubaceae | *Simarouba glauca* DC | Paradise tree | 9 | 0.9 ± 1.7[a] | 40.4 ± 15.0[a] |
| | *Leitneria floridana* Chapman | Corkwood | 9 | 2.7 ± 1.9[a] | 41.6 ± 18.5[a] |
| Aceraceae | *Acer rubrum* L. | Red maple | 4 | 0[a] | 26.6 ± 1.6[a] |
| Magnoliaceae | *Liriodendron tulipifera* L. | Tulip poplar | 4 | 0[a] | 28.7 ± 16.8[a] |
| Anacardiaceae | *Rhus typhina* L. | Staghorn sumac | 4 | 0[a] | 22.3 ± 16.8[a] |
| Leguminosae | *Robinia pseudoacacia* L. | Black locust | 4 | 0[a] | 27.0 ± 5.0[a] |
| Rosaceae | *Prunus serotina* Ehrh. | Black cherry | 2 | 0[a] | 26.0 ± 8.4[a] |

[a] Denotes significant differences ($P \leq 0.05$) between *Ailanthus altissima* and the test species (Student's *t* test).

2 to 3 weeks. The billets and developing larvae were maintained at 22°C. After 2 months, the billets were checked daily to capture emerging adults. Through 2006, 523 F1 generation adults emerged. Generally, their size was smaller than weevils shipped from China. As we refine this rearing method, we will focus on using fewer weevils in the oviposition cages to reduce competition among developing larvae in the billets.

## Adult feeding test

Of the species tested, feeding by *E. brandti* adults occurred only on species in Simaroubaceae. Tree of heaven was highly favored for feeding over the non-target species tested (Table 6). In no-choice tests, adults consumed nearly seven and three times more of tree of heaven foliage than *S. glauca* and *L. floridana*, respectively. No feeding occurred on 15 other non-target species. In two-choice tests, an even less non-target species foliage was consumed. When given a choice of tree of heaven and *S. glauca* or *L. floridana*, *E. brandti* adults consumed 48 and 15 times more tree of heaven foliage than the two non-target species, respectively. No feeding occurred on five species outside of Simaroubaceae (Table 7).

## Billet inoculation assay

Seven *E. brandti* larvae were inoculated into 7.5-cm diameter × 76-cm long billets of tree of heaven, *Robinia pseudoacacia*, *Prunus serotina* and *Citrus aurantifolia* to determine if young larvae (<30 days old, approximately second instar) can survive on plants other than tree of heaven. These preliminary assays were replicated twice, and larvae remained in billets for 30 days. Our results indicate a high level of specificity for tree of heaven. No feeding occurred in species other than tree of heaven, resulting in 100% larval mortality (Fig. 2). This is compared with 36% larval survival in *A. altissima* past the instar when the larvae were inoculated.

# Conclusions

The survey work in Virginia helped characterize tree species associated with tree of heaven, with *Quercus* spp. being the predominant associate regardless of region. The insects found feeding on tree of heaven were of inconsequential value in terms of damaging the weed tree and contributing to its overall control. Rearing studies have improved to the point that a continuous

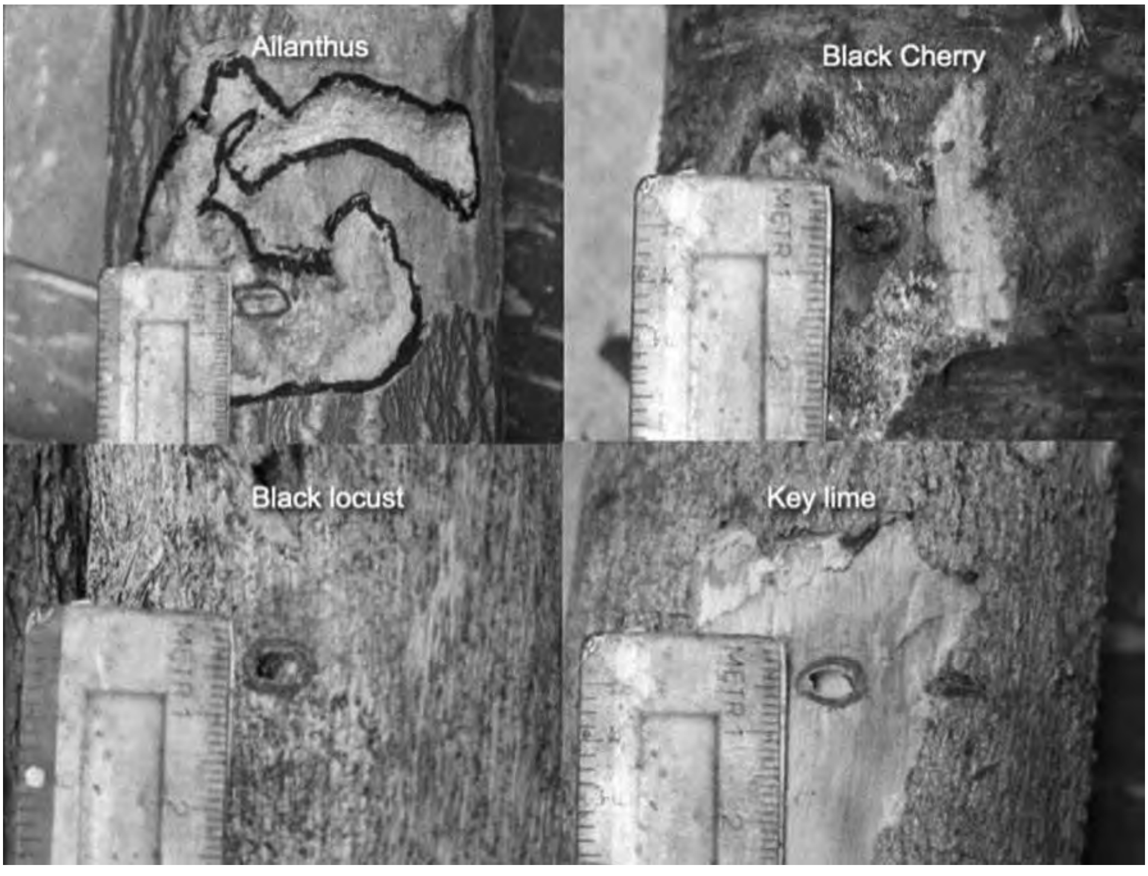


**Figure 2.** Example of *Eucryptorrhynchus brandti* (Harold) larval galleries in each of 4 species tested. Note that galleries were created only in *Ailanthus altissima* (Mill.) Swingle (photo credit, N. Herrick).

colony of *E. brandti* is possible. The use of billets with one end sealed with paraffin and the other sitting in water may be an adequate bioassay for larval survival tests. Preliminary results from the quarantine studies showed that the risk of this beetle attacking non-target trees is minimal. Continued testing is ongoing.

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# The insect fauna of *Chondrilla juncea* L. (Asteraceae) in Bulgaria and preliminary studies of *Schinia cognata* (L.) (Lepidoptera: Noctuidae) as a potential biological control agent

**I. Lecheva,[1] A. Karova[1] and G. Markin[2]**

*Summary*

Between 2001 and 2005, a survey of rush skeletonweed, *Chondrilla juncea* L. (Asteraceae), and its associated insect fauna was conducted in Bulgaria. The weed occurs from sea level to 1200 m mainly on roadsides (47% of populations encountered), disturbed and abandoned farmlands (40%), as well as in orchards, vineyards and fields of wheat, roses and lavender. For the first time in Bulgaria, the insect species associated with the plant were inventoried. A total of 51 insect species were collected, but only four appeared to be specific to the plant. The most dominant species and the one considered most promising as a potential biological control agent was the moth, *Schinia cognata* Fr. (Lepidoptera: Noctuidae). *S. cognata* larvae feed on the reproductive parts of the plant, and during development, one larva can consume 61 to 62 flower buds or seed heads. In Bulgaria, the moth has two generations that overlap, with maximum population densities in July and August. *S. cognata* is widely distributed throughout Bulgaria and was found in high densities in most of the populations of *C. juncea* studied. It was not observed attacking any other native plant or cultivated plants, and preliminary host-range studies of four closely related species indicated that it could only feed and develop on *C. juncea*. *S. cognata* has therefore been selected as a potential biological control agent for possible future introduction in North America.

***Keywords***: *Schinia cognata*, host-range testing, rush skeletonweed, distribution.

## Introduction

Rush skeletonweed, *Chondrilla juncea* L. (Asteraceae), has been accidentally introduced in northwest USA and adjacent Canada, and in Argentina and Australia and in all areas, it has become a major noxious weed. In Australia, a program in the 1970s resulted in the successful introduction of three biological control agents that soon controlled *C. juncea* over most of its range (Cullen and Groves, 1977). A similar program by the Agricultural Research Service of the US Department of Agriculture introduced and established the same three agents in North America and resulted in satisfactory control of the weed in the state of California and some areas of Washington (Piper and Andres, 1995). However, in the cooler interior states of Oregon, Idaho and Montana, the three agents have not given effective control. A new program was implemented to determine if new and more effective biological control agents could be found in Eurasia (Markin and Quimby, 1997). As part of this program, a study on *C. juncea*-associated phytophagous insects to identify potential biocontrol agents was conducted in Bulgaria in 2000 to 2005 (Karova and Lecheva, 2005, Karova, 2006).

## Methods and materials

From 2000 to 2005, field visits were made to all regions of Bulgaria to determine the distribution, main habitats, population densities and phenology of *C. juncea*. When stands of *C. juncea* were encountered, the plants were visually examined, and all associated insects collected. Samples of flower buds, stems and roots were also collected and dissected. Individuals collected as

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larvae were reared in the laboratory using potted *C. juncea* plants or using the appropriate plant part in plastic cages or Petri dishes, and adults that emerged were identified.

From this survey, the most common and destructive insect encountered was the moth, *Schinia cognata* Fr. (Lepidoptera: Noctuidae), which was selected for more detailed studies. Laboratory studies of the development and feeding behaviour of *S. cognata* were conducted at the facility of the Department of Agri-Ecology and Entomology of the Agricultural University of Plovdiv (Bulgaria). Observations on the seasonal population dynamics of *S. cognata* were conducted on two populations near Plovdiv in central Bulgaria in 2003 and 2004, using sweep netting. The sites were visited eight times during each summer, and five batches of 100 plants swept with a 37-cm diameter canvas sweep net. The sampling was carried out primarily to determine the abundance of *S. cognata* adults and larvae, but all other insects collected were also counted to provide an estimate of their relative abundance (percent of all insects collected over the 2 years). To determine the potential host range of *S. cognata*, other plant species of Asteraceae growing adjacent to attacked *C. juncea* plants were searched for *S. cognata* larvae.

Laboratory feeding tests were conducted using field collected early instar larvae or larvae reared from eggs. We determined the development and feeding impact (number of reproductive structures consumed) of larvae held on potted plants and bouquets of plants, covered by transparent plastic screen cages. Choice feeding tests used bouquets of *C. juncea* intermixed with a test plant with their bases wrapped in cotton, placed in small tubes of water and held in plastic screen cages. For no-choice feeding tests, the reproductive parts of the plant being tested were offered to larvae held in a 20 × 1.5 cm glass Petri dish. Reproductive parts were replaced when consumed or wilted until either the larvae pupated or died. All choice and no-choice tests were replicated ten times using one larva each. For these preliminary host tests, four locally available species of closely related Asteraceae were used.

# Results and discussion

## Distribution, habitats, population density and phenology of *C. juncea*

*C. juncea* is widespread throughout Bulgaria and was found in 23 of the 26 regions (=states; Karova and Lecheva, 2005). It occurs mainly on roadsides and disturbed lands and also in orchards, grape vineyards and fields of wheat, lavender and roses (Table 1). It was found from 0 to 1200 m above sea level. The densest populations (50 plants/m$^2$) were observed on abandoned farmland around the cities of Plovdiv, Varna, Ihtiman and Dupnitza and nearly as dense populations (25 to 50 plants per square meter) in Blagoevgrad. Other plants occurring with *C. juncea* were usually other weed species such as *Cichorium intybus* L. (Asteraceae), *Chamomilla recutita* (L.) Rauschert (Asteraceae), *Avena fatua* L. (Poaceae), *Cuscuta* spp. (Convolvulaceae), *Cirsium arvense* (L.) Scop. (Asteraceae), *Centaurea cyanus* L. (Asteraceae) and *Verbascum thapsus* L. (Scrophulariaceae).

**Table 1.** Frequency distribution by habitat of 84 stands of *Chondrilla juncea* found in a survey of Bulgaria between 2001 and 2005 (Karova and Lecheva, 2005; Karova, 2006).

| Habitats in which *Chondrilla juncea* was found | Percent of stands |
|---|---|
| Road sides | 46.75 |
| Abandon farmland | 39.4 |
| Fallow wheat fields | 4.25 |
| Grape vineyards | 4.25 |
| Fields of roses[a] | 1.06 |
| Fields of lavender[a] | 1.06 |
| Other locations | 4.27 |

[a] Fields of ornamental roses (*Rosa* spp.) (Rosaceae) and lavender (*Lavandula officinallis* L.) (Labiatae) are extensively grown in Bulgaria as a source of fragrance.

The vegetative growth of rush skeletonweed in Bulgaria begins at the end of March and the first weeks of April depending on the local climatic conditions and altitude. Flower buds are formed at the beginning of June, and flowering was observed at the end of the same month; by November, the flowering stem had died.

## Phytophagous insects feeding on *C. juncea*

During the 5 years of the survey, a total of over 51 insect species from Coleoptera, Lepidoptera, Heteroptera, Homoptera and Diptera were found associated with *C. juncea*. The species found, their density and seasonal occurrence has been presented elsewhere (Karova and Lecheva, 2005; Karova, 2006). Most of the identified species were moth larvae, leaf beetles, plant bugs and other sucking insects. To a lesser extent, the flower buds, flowers and seed heads of *C. juncea* were damaged by plant bugs, Lepidoptera larvae and beetles. Only two species were recovered from the roots: larvae of the moth, *Bradyrrhoa gilveolella* Tri. (Lepidoptera: Pyralidae), which feeds in a sand-encrusted silken case on the outside of the root, and the beetle *Mordelistena micans* Germ. (Coleoptera: Mordellidae), the larva of which mines down the central core of the root. Most of the species are generalists and thus cannot be considered as potential biocontrol agents. Only four species appeared to have a restricted host range: *B. gilveolella*, *M. micans*, the midge *Cystiphora schmidti* Rub. (Diptera: Cocidomidae) and the flower-feeding moth, *S. cognata* Fr. (Lepidoptera: Noctuidae). *B. gilveolella* and *C. schmidti* have already been studied and introduced as biological control agents in Australia and the USA (Julien and Griffin, 1998; Piper *et al*., 2004). Min-

ing by *M. micans* appears to have no effect on the plant, so we concentrated most of our effort on studying *S. cognata*.

## *S. cognata*

Among all recovered insect species in Bulgaria, the seed-head-feeding moth, *S. cognate*, was the most abundant and damaging and seemed the most promising candidate for biological control of *C. juncea*. It represented 20% to 34% of all the insects collected in sweeping the plants in 2003 and 2004 (Table 2). Field collected larvae of *S. cognata* in flower buds of *C. juncea* were most abundant not only in the vicinity of Plovdiv but were also recovered in most regions of the country. Very little previous information exists on *S. cognata*. According to Nowacki and Fibiger (1996) and Rakosy (1996), it occurs in the European part of the former USSR, Czech Republic, Austria, Hungary, Former Yugoslavia, Greece, Albania and Bulgaria. The first record of *S. cognata* in Bulgaria was from Bahmetev (1902) who found only single individuals. Until now, the moth was considered a rare species. The results of our study show that it has two generations per

**Table 2.** Relative abundance of insects collected on *Chondrilla juncea* by sweep netting over the summer of 2004 at two locations near Plovdiv, Bulgaria. Over 50 species were collected (Karova and Lecheva, 2005; Karova, 2006), but only these were observed or suspected of actually feeding on the plant.

| Genus and species | Family | Feeding site | Percent abundance |
|---|---|---|---|
| **Coleoptera** | | | |
| *Gastrophysa polygoni* L. | Chrysomelidae | Foliage | 0.95 |
| *Cassida nebulosa* L. | Chrysomelidae | Foliage | 1.09 |
| *Clytra novempunctata* L. | Chrysomelidae | Flower | Occ.[a] |
| *Coptocephala rubicunda* Laich | Chrysomelidae | Foliage | Occ. |
| *Coptocephala unifasciata* Scop. | Chrysomelidae | Foliage | Occ. |
| *Cryptocephalus connexus* Ol. | Chrysomelidae | Foliage/Flower | 2.45 |
| *Cryptocephalus sericeus* L. | Chrysomelidae | Flower | 3.59 |
| *Epicometis hirta* Poda. | Scorabaeidae | Flower | 1.63 |
| *M. micans* Germ.[b] | Mordellidae | Roots | 0.85 |
| *Mylalris polymorpha* Pall. | Meloidae | Pollen | 10.17 |
| *Zonabris polymorpha* Pall. | Meloidae | Pollen | 10.76 |
| **Lepidoptera** | | | |
| *Bradyrrhoa gilveolella* Tr.[b] | Pyralidae | Roots | Occ. |
| *Emmelia trabealis* Scop. | Noctuidae | Foliage | 10 |
| *Idaea muricata* Hufn. | Geometridae | Foliage | 1.24 |
| *Melitaea didyma* Esp. | Nymphalidae | Flower | 10.26 |
| *Plusia gamma* L. | Noctuidae | Flower | 0.67 |
| *S. cognata* Fr. | Noctuidae | Flower bud | 27.15 |
| *Simira nervosa* (Den. & Schiff.) | Noctuidae | Foliage | 0.81 |
| **Heteroptera** | | | |
| *Adelphocorvis lineolatus* Goez. | Miridae | Sap feeding | 4.29 |
| *Brachycoleus decolar* Renter | Miridae | Sap feeding | 1.25 |
| *Dolycoris baccarum* L. | Pentatomidae | Sap feeding | 3.41 |
| *Eurybema oleraceae* L. | Pentatomidae | Sap feeding | Occ. |
| *Eurydema ornata* L. | Pentatomidae | Sap feeding | Occ. |
| *Lygus equestris* L. | Lygaeidae | Sap feeding | Occ. |
| *Lygus pratensis* L. | Lygaeidae | Sap feeding | Occ. |
| *Lygus rugulipennis* Popp. | Lygaeidae | Sap feeding | Occ. |
| *Syramastes rhombeus* L. | Lygaeidae | Sap feeding | 1.46 |
| **Homoptera** | | | |
| *Aphis* spp. | Aphididae | Sap feeding | 2.45 |
| *Philaenus spumarius* L. | Cereopidae | Sap feeding | 5.45 |
| **Diptera** | | | |
| *C. schmidti* Rub.[b] | Cecidomiidae | Under cuticle | Occ. |
| *Dasyneura* sp. | Cecidomiidae | Flower | Occ. |

[a] Occ. = Occasionally found, but were generally rare or found for only a very short period during the summer

[b] These insects were rarely collected as adults during sweep netting, but their larvae were found on or in the plants.

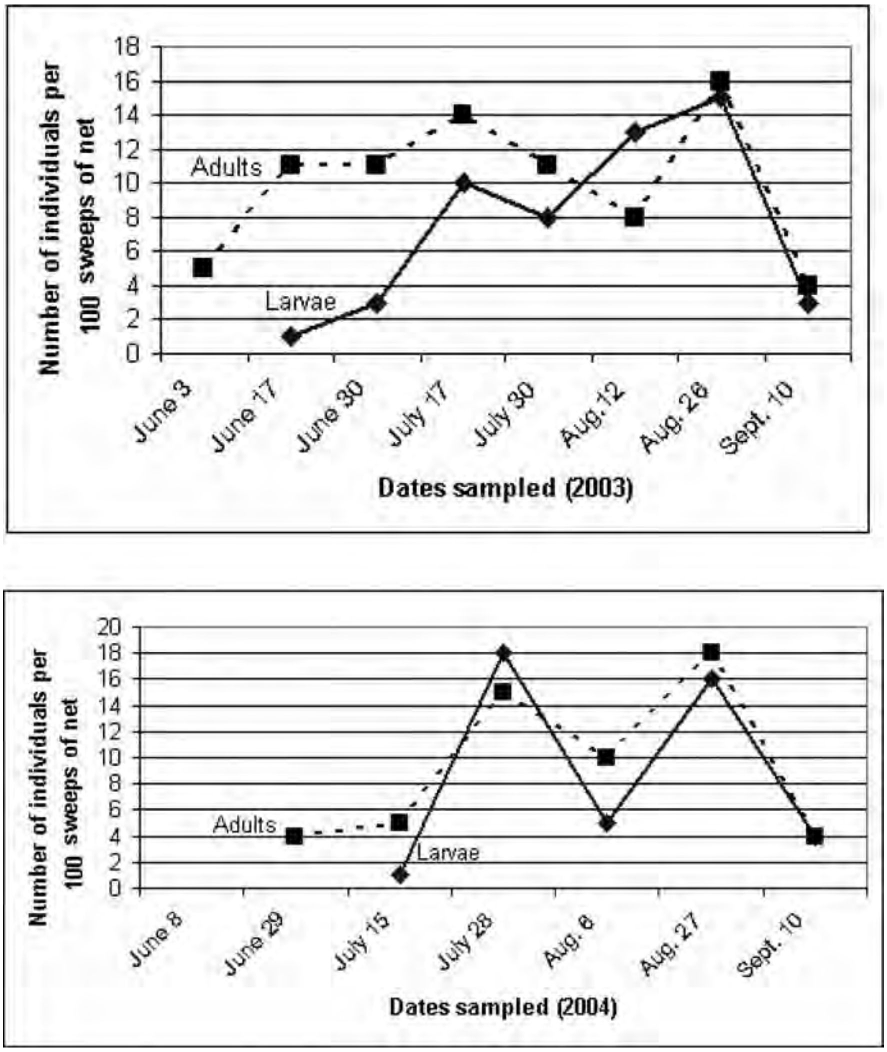


**Figure 1.** Season population density (determined by 5 × 100 sweeps with a collection net) of larvae and adults of *Schinia cognata* near Plovdiv, Bulgaria in 2003 and 2004.

year that overlap, with maximum population densities in July and August (Fig. 1). The moth overwinters as a pupa, and emergence begins in early June. The females lay their eggs singly on the upper parts of the stems, flower buds and flowers of the host plant beginning in mid-June. The eggs begin to hatch in the first weeks of July. In the two populations examined near Plovdiv, the highest density of larvae was observed in mid-July and mid-August. The larvae feed on the reproductive parts of *C. juncea*, i.e. flower buds, flowers and seed heads. Observations under laboratory conditions showed that, for full development, one larva consumes 61 to 62 flower buds, flower heads or seed heads.

During our investigations, the moth was not encountered on other related weed species or cultivated plants growing in the vicinity of attacked *C. juncea* plants. Laboratory feeding tests confirmed its host specificity. Four closely related species of Asteraceae were used for both choice and no-choice trials: *C. intybus* L., *Lactuca serriola* L., *Lactuca sativa* L. (domestic lettuce), and *Sonchus oleraceus* L. In all choice trials, the larvae fed only on *C. juncea*, and there was no feeding on the four tested plants. In the no-choice trials, where the larvae were forced to feed on plants different from *C. juncea*, some larvae attempted to feed but none survived to pupate. On *C. juncea*, larvae development was normal; most of them pupated and adults emerged successfully.

## Conclusion

Among the insect species found feeding on *C. juncea* in Bulgaria, the dominant species was the moth *S. cognata*. The larvae feed on the reproductive parts of the plant and were observed to cause extensive damage in the field. *S. cognata* is widely distributed, occurs at high densities in all *C. juncea* population studies and was not recorded or observed feeding on other, local or cultivated plants. It could not be reared on four closely related plant species and is therefore considered a po-

tential biocontrol agent. Arrangements have been made to ship colonies of *S. cognata* to a plant containment facility in Bozeman, Montana in the USA where it can undergo more extensive host testing on North American crops and native species.

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# Biological control of aquatic weeds by *Plectosporium alismatis*, a potential mycoherbicide in Australian rice crops: comparison of liquid culture media for their ability to produce high yields of desiccation-tolerant propagules

**C. Moulay,[1] S. Cliquet,[1] K. Zeeshan,[1] G.J. Ash[2] and E.J. Cother[3]**

*Summary*

A methodology to develop a stable, effective *Plectosporium alismatis* (Oudem.) Pitt, Gams and Braun [syn. *Rhynchosporium alismatis* (Oudem) J.J. Davis] mycoherbicide is currently being investigated. We compared a nitrate–malt extract medium liquid culture medium to other liquid media for their ability to support high conidial and chlamydospore yields and subsequent tolerance of conidia and chlamydospores to air-drying. When grown in a casamino acids–glucose-based liquid medium, *P. alismatis* developed hyphae and produced high yields of conidia (1 × $10^7$ conidia per millilitre) and dry weights (220 mg dry weight per erlen), while no chlamydospore was formed. In a nitrate–glucose-based medium, growth was poor, *P. alismatis* producing aggregated hyphae that contained 6.5 × $10^4$ chlamydospores per millilitre. The addition of nitrate in the casamino acids–glucose-based medium restored partially chlamydospore formation (1 × $10^4$ chlamydospores per millilitre). Chlamydospores and conidia were air-dried and stored at 25°C. No conidia germinated after 40 days storage, while 50% to 20% chlamydospores, respectively, produced in a nitrate–malt extract medium or in nitrate–glucose medium, remained viable after 120 days storage.

***Keywords***: fermentation, air-drying, storage, chlamydospores, conidia.

## Introduction

The endemic fungus, *Plectosporium alismatis* (Oudem.) Pitt, Gams and Braun [syn. *Rhynchosporium alismatis* (Oudem) J.J. Davis] (Pitt *et al.*, 2004) is being developed as a mycoherbicide (Crump *et al.*, 1999) for the control of starfruit and other closely related weed species (Jahromi *et al.*, 2001). The fungus sporulates abundantly on solid media (Jahromi *et al.*, 1998) and is able to infect host species (Lanoiselet *et al.*, 2001), leading to reduced biomass of the weed or to reduced seed set (Fox *et al.*, 1999).

A culture production method for the development of *P. alismatis* mycoherbicide is currently being investigated. *P. alismatis* produces high numbers of conidia in most liquid media; chlamydospore formation also occurs in a liquid standard medium based on the Czapex–Dox composition, in which nitrogen and carbon are provided by sodium nitrate (3 g $l^{-1}$) and malt extract (2.2 g $l^{-1}$; Cliquet *et al.*, 2004).

We modified the carbon and the nitrogen sources and concentrations of the nitrate–malt extract medium to investigate the impact of nitrogen and carbon sources on both conidia and chlamydospore inductions. Shelf-life of air-dried conidia and of air-dried chlamydospores produced in modified carbon and nitrogen

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sources was investigated and compared to shelf-life of air-dried propagules produced in the nitrate–malt extract medium.

# Materials and methods

## Isolate

*P. alismatis* RH 145 (DAR 73154) was isolated from *Damasonium minus* (R.Br) Buch., which was obtained from the culture collection of the New South Wales Department of Primary Industries. Stock cultures were maintained in a soil/sand mixture.

## Inoculum production

Sub-cultures on potato dextrose agar (PDA, Difco, Detroit, MI, USA) were sampled from the soil and sand mixture and renewed every year. From these sub-cultures, conidia were inoculated on PDA plates and incubated at 25°C. Four-day-old Petri dishes of the fungus were washed with distilled water to produce conidial suspensions for liquid culture as described hereafter.

## Media composition

The basal mineral composition of nitrate–malt extract medium and defined medium was derived from a Czapex–Dox composition containing: 1.0 g $K_2HPO_4$; 1.0 g $MgSO_4.7H_20$; 0.5 g KCl; 0.018g $Fe_2SO_4.7H_2O$ in 1 l deionized water. The nitrate–malt extract medium contained 3 g $NaNO_3$ (Sigma Chemicals, St. Louis, MO, USA ) and 2.2 g malt extract (Amyl Media).

In the defined medium, malt extract was replaced by 2.4 g $l^{-1}$ glucose at the carbon concentration (920 mg C $l^{-1}$) provided by malt extract. The 3.12 g $l^{-1}$ $NaNO_3$ provided the same nitrogen content (0.5 g N $l^{-1}$) than in the nitrate–malt extract medium. Bacto yeast nitrogen base without amino acids and $(NH_4)_2SO_4$ (Difco) was provided as a nitrogen-free vitamin source (0.17 g $l^{-1}$). As the organic nitrogen source, 0.5 g N $l^{-1}$ technical casamino acids (Difco) was used. In shelf-life experiments, propagules were produced in media containing 3.68 g C $l^{-1}$ and 1 g N $^{-1}$.

## Growth and harvest

All cultures of 100 ml were placed in 250-ml flasks (Bellco Glass, Inc, Vineland, NJ, USA), inoculated with $4 \times 10^5$/ml conidial suspension and incubated at 25°C on a rotary shaker incubator (Certomat BS-1 Braun Biotech International, Germany) at 100 rpm for 7 days for growth experiments or for 4 days for drying and storage experiments. Cultures were vacuum-filtered on 110 mm ∅ cellulose filter papers (Whatman plc, Brentford, UK). Filtered cultures were rinsed with 50 ml deionized water and allowed to dry on the bench top until constant weight. Dry mats were weighed and re-suspended in 50 ml distilled water. The suspension was fragmented in a Potter homogenizer (Fisher). Spore counts were performed using a haemocytometer.

## Chlamydospore and conidial germination

Drops of the propagule suspension were placed on four 2-$cm^2$ pieces of cellophane on the surface of water agar plates. Cellophane pieces were removed after 12 h at 25°C and germination evaluated microscopically using lactophenol cotton blue as previously described (Cliquet *et al.*, 2004).

## Statistical analysis

All growth experiments were performed using duplicate or triplicate flasks, and all experiments were repeated at least once. Statistical analysis of variance was performed. For data not suitable for analysis of variance, standard errors values were estimated as a measure of variance.

# Results and discussion

*P. alismatis* produced significantly higher yields of conidia when grown in casamino acids ($1 \times 10^7$conidia per millilitre) compared to conidial yields produced in sodium nitrate ($2 \times 10^5$conidia per millilitre; Fig. 1). When nitrate was the sole nitrogen source, $6.5 \times 10^4$ chlamydospores per millilitre were observed; however, the addition of sodium nitrate to the medium containing casamino acids resulted in the production of less chlamydospores ($1 \times 10^4$ chlamydospores per millilitre; Fig. 1).

When grown in a medium containing casamino acids, *P. alismatis* produced numerous hyphae resulting in high dry weights with a maximum of 220 mg dry weight per erlen, compared to low dry weights (80 mg per erlen) obtained when sodium nitrate was the sole nitrogen source (Fig. 2). Homogenization of cultures showed that chlamydospores were mainly formed inside these aggregates.

In our culture conditions, replacing sodium nitrate by casamino acids as the sole nitrogen source and at the same nitrogen content improved growth as expressed by the high conidial yields and dry weights. Organic nitrogen provided by casamino acids was probably utilized preferentially to inorganic nitrogen by *P. alismatis*, as previously reported for a majority of filamentous fungi (Garraway and Evans, 1984). The absence of chlamydospore formation in these conditions is likely the consequence of the addition of organic nitrogen.

In filamentous fungi, chlamydospore production may vary considerably depending on the nutritional environment, i.e. a nutrient excess or starvation conditions (Gardner *et al.*, 2000). In our culture conditions, starvation due to a lack of organic nitrogen is likely responsible for chlamydospore formation.

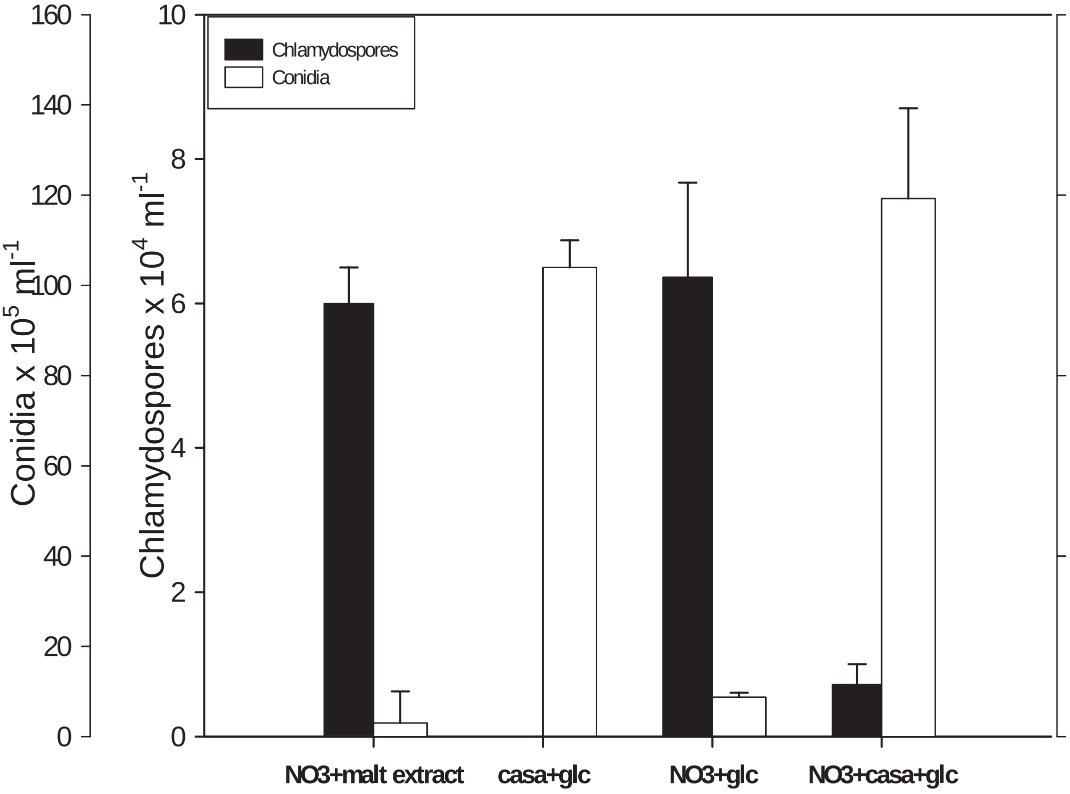


**Figure 1.** Impact of the carbon and nitrogen sources on *Plectosporium alismatis* conidial and chlamydospore production. $NaNO_3$ + malt extract: sodium nitrate: 3 g $l^{-1}$; malt extract: 2.2 g $l^{-1}$; $NaNO_3$ + GLC: sodium nitrate + glucose (1); CA+GLC: casamino acids + glucose (2); $NaNO_3$+CA+GLC: sodium nitrate + casamino acids + glucose (3); In (1), (2) and (3), glucose provides 0.92 g C $l^{-1}$; nitrogen sources provide each 0.5 g N $l^{-1}$.

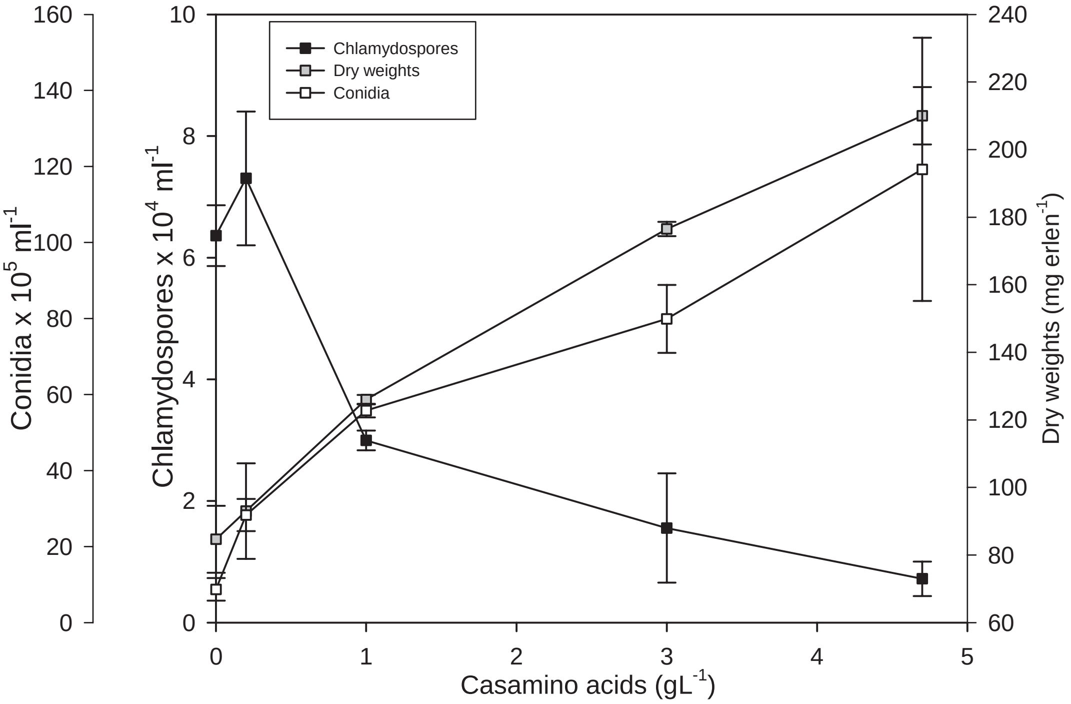


**Figure 2.** Influence of casamino acids concentration on production of chlamydospores and conidia by *Plectosporium alismatis* (Na nitrate: 0.5 g N $l^{-1}$; casamino acids 4.7g $l^{-1}$ = 0, 5g N $l^{-1}$).

Conidia and chlamydospores produced in nitrate–malt extract or nitrate–glucose were air-dried, and germination was estimated during storage at 25°C (Fig. 3).

The type of propagules, i.e. conidia or chlamydospores, had a significant impact on survival rate (Fig. 3). No conidia germinated after 40 days storage, while 50 to 20% chlamydospores, respectively, produced in a nitrate–malt extract medium or in nitrate–glucose medium remained viable after 120 days storage (Fig. 3).

Microscopic observation showed that some chlamydospores produced in nitrate–glucose medium sporulated through a microcycle conidiation.

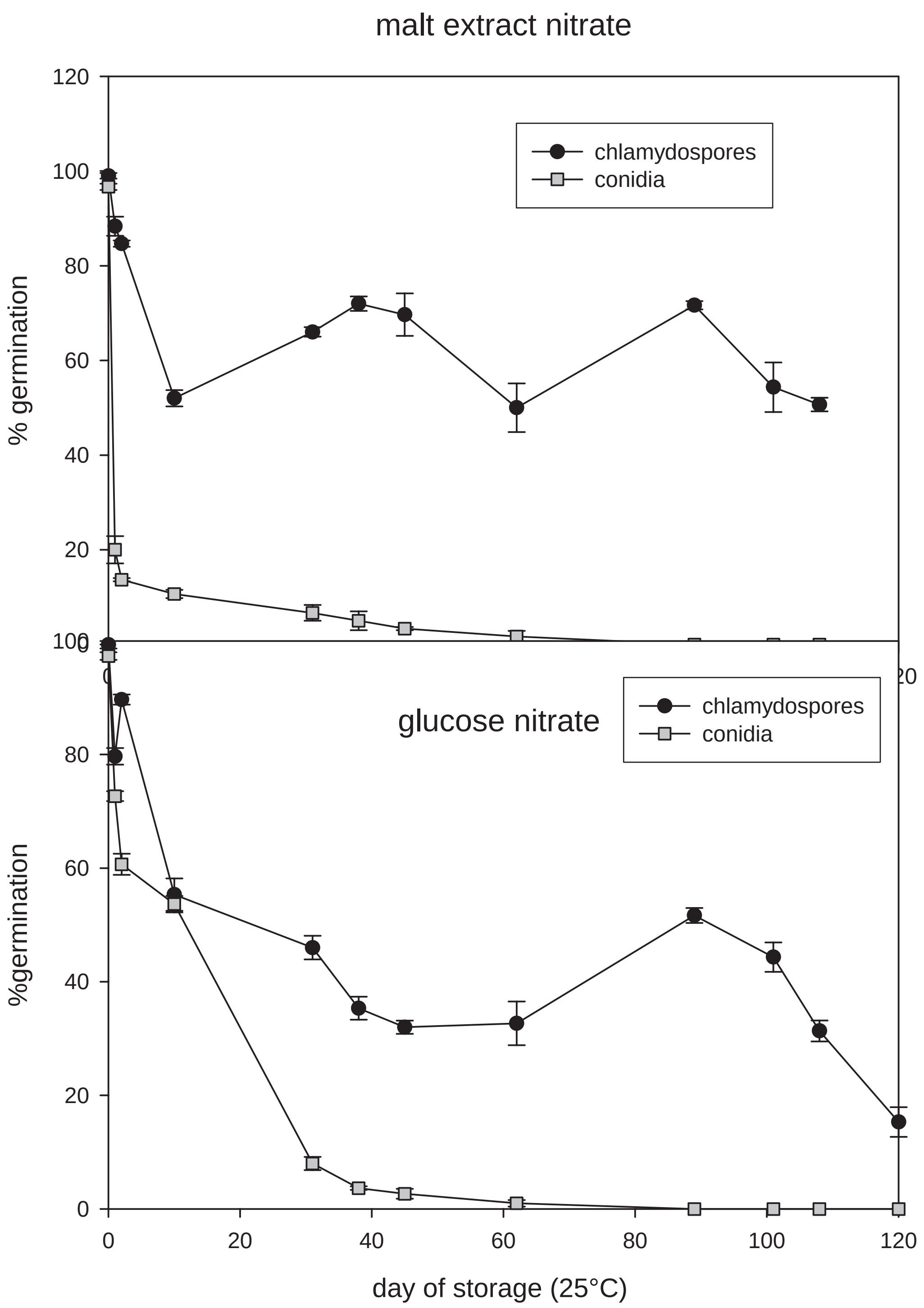


**Figure 3.** Shelf-life at 25°C of air-dried *Plectosporium alismatis* conidia and chlamydospores. *Plectosporium alismatis* was grown in a nitrate–malt extract medium (8.8 g $l^{-1}$ malt extract, 5.74 g $l^{-1}$ sodium nitrate) or in a nitrate–glucose medium (6 g $l^{-1}$ sodium nitrate, 10 g $l^{-1}$ glucose) and harvested after 7 days growth.

## Conclusion

This work shows that numerous factors are to be investigated to develop a stable mycoherbicide. Survival during storage depends upon the type of propagules produced and upon the culture conditions during growth. Moreover, the microcycle conidiation observed during germination experiments may allow the fungus to extend rapidly and colonize aquatic weeds effectively. As a conclusion, chlamydospores may be promising stable propagules compared to conidia, although the nutritional conditions impact these qualities.

More work needs to be done to consider as many parameters (physical, chemical and morphological) as possible in an experimental design for the selection of factors that impact chlamydospore formation and tolerance to drying.

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# Feeding and oviposition tests refute host–herbivore relationship between *Fragaria* spp. and *Abia sericea*, a candidate for biological control of *Dipsacus* spp.

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*Summary*

Two species of teasels, *Dipsacus fullonum* L. and *Dipsacus laciniatus* L. (Dipsacales: Dipsacaceae), have become invasive in the USA and are targets of a classical biological control programme. The sawfly, *Abia sericea* (L.) (Hymenoptera: Cimbicidae), was identified as a teasel biological control candidate, but reports in the literature raised concerns about the possibility of non-target effects on members of the genus *Fragaria* L. Oviposition tests and multiple- and no-choice feeding tests were conducted on *A. sericea* to test the suitability of three *Fragaria* spp. (*Fragaria* × *ananassa* Duchesne, *Fragaria vesca* L. and *Fragaria viridis* Duchesne) as host plants. In oviposition tests, males were paired with females in cages containing a *D. laciniatus* plant and plants of the three *Fragaria* spp. In 16 replicates, all eggs were laid in *D. laciniatus* leaves, except in one replication in which two eggs were laid in a leaf of *F. viridis* (vs >100 eggs in *D. laciniatus* leaves by the same female and >1000 eggs by all females). From these two eggs, one larva hatched, which fed only on *D. laciniatus* in a choice test and died before pupating. In no-choice feeding tests on the three *Fragaria* spp., larvae of first, second, third and fourth instars were infested in separate tests, and no feeding, tasting or damage was observed on any *Fragaria* spp. in any replicate; all larvae died. In multiple-choice feeding tests, first, second, third and fourth instar larvae were reared to pupation. Larvae of all instars fed exclusively on *D. laciniatus*, while no feeding attempts, tastes or feeding damage was observed on any *Fragaria* spp. plant. Entomology and agricultural literature were thoroughly reviewed, and the connection between *Fragaria* and *A. sericea* was traced to two brief anecdotal mentions that were widely repeated but never supported by collection or bioassay data. The tests and other evidence presented in this paper refute the idea that *Fragaria* spp. are suitable hosts for *A. sericea*.

***Keywords***: teasel, invasive plants, strawberry.

## Introduction

Two invasive teasels of European origin, *Dipsacus fullonum* L. and *Dipsacus laciniatus* L., are emerging as problem weeds in various parts of North America, particularly in non-agricultural habitats (Sforza, 2004). Either or both species occur in 43 states (Singhurst and Holmes, 2001; USDA, 2005; Rector *et al.*, 2006) and in several Canadian provinces (Werner, 1975). Five states (Colorado, Iowa, Missouri, New Mexico and Oregon) have declared teasels noxious (USDA-NRCS, 2005). They are also listed as invasive by 11 other states and as affecting natural areas in 14 states and four national parks (USDI-NPS, 2005). This combined status and other factors led to the initiation of a US-government-sponsored biological control programme targeting *Dipsacus* spp. in 2003.

The genus *Dipsacus* L. is in the family Dipsacaceae, an exclusively Old World family with no species native to the Western Hemisphere (Verlaque, 1985) and no members of significant economic importance (Bailey, 1951). Thus, in selecting biological control candidates (BCCs) to combat invasive teasels in North America, those restricted to feeding on hosts within the family Dipsacaceae should be specific enough to avoid non-target concerns (Wapshere, 1974; Rector *et al.*, 2006). However, for any teasel BCC, the list of plants tested will include many species outside the family Dipsacaceae, including economic and rare or threatened species, as well as those occupying similar ecological

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niches, those sharing similar life histories, plant architecture and secondary chemistry, and those with published associations with the BCC (Rector *et al.*, 2006).

*Abia sericea* (L.) (Hymenoptera: Cimbicidae) is a sawfly that is native to Europe and is a candidate for biological control of invasive teasels in the USA. The most recent, comprehensive host-range records for *A. sericea* (Liston, 1995, 1997; Taeger *et al.*, 1998) list two host species in the family Dipsacaceae [*Knautia arvensis* (L.) Coult. and *Succisa pratensis* Moench], plus the genus *Fragaria* L., which is in the family Rosaceae and includes cultivated and wild strawberries.

In assessing *A. sericea* as a BCC of teasels, it was necessary to start by testing the suitability of *Fragaria* as a host, to either confirm or refute the existing herbivore–host records. Any amount of *A. sericea* larval feeding on *Fragaria* plants, particularly on cultivated strawberry (*Fragaria* × *ananassa* Duchesne), would immediately disqualify it as a BCC of teasel. It was also necessary from the start to confirm the use of *D. laciniatus* as a host plant suitable for complete development by *A. sericea*, as this relationship had only previously been observed in the field without further testing (Rector *et al.*, 2006; Rector *et al.*, unpublished data).

The purpose of this paper is to report the successful rearing of *A. sericea* on *D. laciniatus* and to refute the published herbivore–host relationship between *A. sericea* and *Fragaria* spp.

# Materials and methods

## Literature survey

An intensive literature search was conducted to determine the origin(s) of reports of a herbivore–host relationship between *A. sericea* and *Fragaria* spp. An attempt was made to identify the original source literature behind this alleged association and to gauge its plausibility. Literature surveyed included various entomological texts, articles and monographs. Most of these were on the subject of basic sawfly (Hymenoptera: Symphyta) biology and taxonomy (e.g. André, 1879; Stein, 1883; Cameron, 1890; Dalla Torre, 1894; Konow, 1901; Enslin, 1917; Berland, 1947; Lorenz and Kraus, 1957; Ermolenko, 1972; Vasilev, 1978; Wright, 1990; Magis, 2001). Other sources included sawfly host-range compendia (Liston, 1995, 1997; Taeger *et al.*, 1998), work specific to *Abia* spp. (Kangas, 1946) and a general entomology text (Krishtal, 1959). In addition, pest management literature for cultivated strawberries was surveyed to determine whether *A. sericea* is considered a pest to commercial strawberry growers in its native range in Europe.

## Insect material

On 4 September 2005, 29 late-instar larvae of *A. sericea* were collected from the wild on *D. laciniatus* in northern Bulgaria at two locations in the vicinity of Pleven (43°24.26′N, 24°28.76′E and 43°14.16′N, 24°18.24′E) and one location near Lovech (43°04.12′N, 14°44.09′E). All larvae from these three sites were grouped together and reared to pupation under ambient climatic conditions near Plovdiv, Bulgaria (42°08.64′N, 24°43.81′E). On 29 June 2006, adult females were collected from *D. laciniatus* plants in the field near Sofia (42°3757 N, 23°303427 E). Oviposition tests and feeding tests were conducted using all of these insects and their progeny.

## Test plants

The *D. laciniatus* plants used in host-specificity testing were either grown from seed gathered from wild plants in Bulgaria or were transplanted from fallow fields near Plovdiv (42°08.64′N, 24°43.81′E). The test plants of the wild *Fragaria* spp. (*Fragaria vesca* L. and *Fragaria viridis* Duchesne) were dug up from the wild on a mountainside near Plovdiv (41°53.80′N, 25°20.07′E), transferred to pots and identified to species with a key (Markova, 1973). Cultivated strawberry (*F.* × *ananassa*) plants were vegetatively propagated from 6-year-old plants.

## Oviposition tests

**Test 1:** On 11 Oct 2005, a male and three female *A. sericea* adults were put in a large cage (40 × 20 × 40 cm, made from clear, plastic panels with fine nylon mesh tops) with two *D. laciniatus* and two *F.* × *ananassa* plants. At 8:30 A.M. on 12 October, the teasel plants were removed from the cages, leaving only the strawberry plants. At 4:30 P.M. on the same day, the teasel plants were returned to the cages. The insects were then left in the cage with both plant species until they died. A 5% sugar solution was provided for the insects with a cotton wick from which to feed.

**Test 2:** Pairs of newly emerged adults were released into small plastic cages (20 × 20 × 30cm) with one potted plant each of *D. laciniatus*, *F.* × *ananassa*, *F. vesca* and *F. viridis*. Cages were kept in an insectary at approximately 22/15°C, day/night, and 16 h daylight. The adults were kept in the cages until they died, after which the plants were removed from the cages and examined with a magnifying glass for eggs laid, and this number was recorded for each plant species. A total of 16 replications were conducted during the period 1 April to 1 Aug 2006.

## No-choice larval feeding tests

No-choice feeding tests were conducted in small plastic cages. In each cage, one plant of each of the three *Fragaria* spp.—*F. vesca*, *F. viridis* and *F.* × *ananassa*—were exposed to six larvae of first instar of *A. sericea*. Four replications were carried out, while one cage with only *D. laciniatus* plants and six larvae of the

same instar from the same cohort was set up as a control. Larvae were checked every 48 h whereupon the number of dead larvae was recorded. The same procedure was followed with four replicates each of second, third and fourth instar larvae that had been reared on *D. laciniatus* to that stage.

## Multiple-choice larval feeding tests

The tests were carried out by placing six *A. sericea* larvae of the same instar into a small cage with one potted plant each of *D. laciniatus*, *F. vesca*, *F. viridis* and *F.* × *ananassa* whose leaves were all touching. To begin the test, two larvae were placed on each of the three *Fragaria* spp. plants, after which they were free to move to other plants. The experiment was repeated three times: from 24 April to 16 May 2006 with seven replications each of larvae from second, third and fourth instars; from 17 May to 15 June with seven replications each of larvae from first to fourth instars and from 7 July to 5 August with eight replications each of larvae from first to fourth instars. The larvae were monitored daily, and the number of dead larvae was recorded. The experiment was terminated after the pupation of the last larva. *D. laciniatus* plants were replaced as needed.

# Results

## Literature survey

There appear to have been only two direct reports of *Fragaria* spp. recorded as a host plant for *A. sericea*, with all other such reports in the literature referring either to one of these two original reports or to others that have, in turn, cited the original two. The first report (Konow, 1901) simply lists *A. sericea* as occurring "on *S. pratensis* and *F. vesca*" while including neither a reference nor any specific collection data nor any information regarding feeding nor rearing experiments. The second independent report alleging an association between *A. sericea* and *Fragaria* comes from a Ukrainian general entomology text by Krishtal (1959) that covers all insects in all orders occurring in that country. This book declares that *A. sericea* is 'polyphagous', although the only food plants listed are two *Fragaria* spp. that are native to Ukraine: *F. vesca* and *F. viridis*, and there is no collection or feeding/rearing information provided. This alleged host–herbivore connection has been repeated many times throughout the entomological literature, particularly in literature pertaining to Symphyta in general (e.g. Enslin, 1917; Ermolenko, 1972; Liston, 1995, 1997; Taeger *et al.*, 1998) or the genus *Abia* in particular (e.g. Kangas, 1946). However, recent reports have cast doubt on this connection. Indeed, Taeger *et al.* (1998) stated explicitly "*Fragaria* is surely not a food plant [for *Abia sericea*] under field conditions." However, they presented no reference or data to support this statement and left *Fragaria* on their host–plants list for *A. sericea*. The Krishtal (1959) information in particular has been characterized as 'unreliable' (A. Taeger, personal communication).

In addition, if *A. sericea* were in fact a herbivore of *Fragaria* spp., it is likely that it would be of concern to commercial strawberry (*F.* × *ananassa*) production within its native range. However, *A. sericea* is not listed as a pest of strawberry in current pest management literature in Bulgaria (Harizanov and Harizanova, 1998), England (Marks, 2008) or France (Lamarque and Bossennec, 2001), three strawberry-producing nations within the native range of *A. sericea*. Taken together, these various lines of evidence provide no support whatsoever to the records by Konow (1901) and Krishtal (1959) suggesting that *Fragaria* spp. are host plants for *A. sericea*.

## Oviposition tests

**Test 1:** A total of 64 eggs were laid on *D. laciniatus* plants by the three *A. sericea* females before the teasel plants were removed from the cage (Figs. 1 and 2). No eggs were laid on the strawberry plants, neither in the presence of the teasel plants nor in their absence. After the teasel plants were returned to the cage, an additional 26 eggs were laid on them.

**Figure 1.** *Abia sericea* female ovipositing into the leaf margin of a *D. laciniatus* plant.

**Figure 2.** *Abia sericea* eggs (along leaf margins) and neonate larvae on a leaf of *Dipsacus laciniatus*.

A difference in female *A. sericea* behaviour was observed that coincided with the presence or absence of the teasel plants in the cage. The females moved restlessly over the surface of the *D. laciniatus* leaves, raising and lowering their abdomens and periodically piercing the leaf epidermis with their ovipositors. This behaviour was not observed when they were on strawberry leaves. When the teasel plants were absent, leaving only the three *Fragaria* spp. plants, the females were no longer active at all. This indifferent behaviour did not appear to correlate with their only spending daylight hours alone with the strawberry plants, as oviposition by *A. sericea* and its associated behaviour was observed on *D. laciniatus* during daylight hours. There appeared to be a stimulus to this oviposition-associated behaviour that was linked to the presence of *D. laciniatus* plants and a lack of such stimulus from the strawberry plants.

Oviposition occurred along the margins of the *D. laciniatus* leaves, with the eggs deposited just beneath the epidermal layer of either the upper or lower leaf surface (Figs. 1 and 2). None of the adults were observed feeding on the sugar solution, consistent with the observation by Vasilev (1978) that most Cimbicidae adults do not require such food.

**Test 2:** The presence of *D. laciniatus* plants did not stimulate oviposition on adjacent strawberry plants or on other surfaces such as the walls of the cage, with only one exception. A total of 16 females laid 936 eggs, 934 of which were laid in *D. laciniatus* leaves (99.8%), while two were laid by one female in a *F. viridis* leaf (0.2%). This same female also laid >100 eggs in *D. laciniatus* leaves during the same trial. Only one of the eggs laid in the *F. viridis* leaf hatched, and the larva died before pupation after feeding exclusively on *D. laciniatus* leaves despite hatching from a *F. viridis* leaf and always having all three *Fragaria* spp. available as food options. There were no eggs laid on any of the *F. vesca* or *F.* × *ananassa* leaves in any of the replications.

## No-choice larval feeding tests

No feeding, feeding attempts or evidence of feeding was observed on any of the *F. vesca*, *F. viridis* or *F.* × *ananassa* leaves in any of the cages (Table 1). Most of the first-instar larvae died after 1 or 2 days, apparently of starvation. The second-instar larvae died 1 to 4 days after their release into the cage. As the instar of the larvae at the beginning of the test increased, the survival period to death also increased. The third-instar larvae died within 5 days (most after 3 to 4 days), and the fourth-instar larvae were all dead by the eighth day (most after 4 or 5 days) without access to *D. laciniatus* plants as food. In contrast, only one second-instar larva died feeding on *D. laciniatus* in a control cage. All other control larvae of all instars (23 in total) fed normally and survived to pupation.

**Table 1.** Summary of oviposition and choice and no-choice feeding experiments of *Abia A. sericea* on *Dipsacus laciniatus*, *Fragaria* × *ananassa*, *F. vesca* and *F. viridis*.

| Test type | Number of replicates | No. eggs laid/larvae feeding on each host plant | | | |
|---|---|---|---|---|---|
| | | *D. laciniatus* | *F.* × *ananassa* | *F. vesca* | *F. viridis* |
| Oviposition, Test 1 | 1 | 90 | 0 | 0 | 0 |
| Oviposition, Test 2 | 16 | 934 | 0 | 0 | 2 |
| Larval feeding, no-choice | 16 | 96 | 0 | 0 | 0 |
| Larval feeding, free-choice | 81 | 486 | 0 | 0 | 0 |

### Multiple-choice larval feeding

A total of 486 *A. sericea* larvae fed exclusively on the teasel plants until they either pupated or died. They did not feed at all on the *F. viridis*, *F. vesca*, or *F.* × *ananassa* plants, and no tasting attempts were observed. Most of the larvae feeding on *D. laciniatus* developed normally and pupated (88.6%). Of the larvae that died while feeding on *D. laciniatus*, some showed symptoms of virus infection (Lacey and Brooks, 1997). A virus was identified from some of these *A. sericea* cadavers as belonging to the family Iridoviridae. These same symptoms were not observed in any of the larvae that died on *Fragaria* spp. in the no-choice tests.

## Discussion

*Fragaria vesca* and *F. viridis*, two species of wild strawberry, have been recorded in the entomological literature as hosts for the European sawfly *A. sericea* (Konow, 1901; Krishtal, 1959). Doubt has been cast on this insect–plant association (Taeger *et al.*, 1998; A. Taeger, personal communication), but to date, there have been no data to either confirm or refute these reports.

The phylogenetic evidence suggests that such a connection is unlikely. If the inclusion of *Fragaria* as a host of *A. sericea* were accurate, this would represent the only known host plants outside the closely related families Caprifoliaceae and Dipsacaceae for any species in the genus *Abia* (Taeger *et al.*, 1998). *Fragaria* is in the family Rosaceae, which is not at all closely related to Dipsacaceae (APG II, 2003). Although host relationships among herbivores do not always follow plant phylogeny, this evidence is not trivial.

Cultivated strawberry, *F.* × *ananassa*, is a hybrid of two New World species, *Fragaria chiloensis* (L.) P. Mill. and *Fragaria virginiana* Duschene (Hokanson *et al.*, 2006), and thus neither the hybrid plant nor its progenitors would have had any exposure to *A. sericea* before the introduction of the two progenitor species into Europe in the 19th century and the subsequent creation of the hybrid (Hokanson *et al.*, 2006).

In an oviposition test with three gravid females, eggs were laid on only *D. laciniatus*. In a second oviposition test with 16 females, a total of 934 eggs (99.8%) were laid in the leaves of *D. laciniatus* plants, while two eggs were laid in a *F. viridis* leaf (0.2%). Possible explanations for the laying of these two eggs on a non-host plant are central nervous system excitation or sensitation, as described by Marohasy (1998), or the lack of appropriate leaf-edge oviposition sites available on the *D. laciniatus* plant in the cage (whose leaves were more or less lined with eggs by the end of the trial). In no-choice feeding studies presented in this paper, all insects died without leaving any trace of feeding or attempted feeding. In control cages in which larvae from the same cohorts as the insects tested on *Fragaria* spp. were feeding exclusively on *D. laciniatus*, 23 of 24 developed to pupation.

Given that the purported associations between *A. sericea* and *Fragaria* spp. have been shown in this study to be invalid and given that all confirmed hosts of A. *sericea* belong to the family Dipsacaceae, there is no reason to suspect that *A. sericea* will be a threat to *F.* × *ananassa*. The absence of concern for *A. sericea* in European strawberry pest management literature supports this assertion.

## Conclusions

There was no evidence to support a herbivore–host relationship between *A. sericea* and any *Fragaria* spp. These results, combined with evidence from the entomological and strawberry pest management literature and the lack of any comparable data or evidence in the literature supporting such an insect–plant association, strongly suggest that existing records of such a relationship are erroneous. At the least, it can be confidently stated that the populations of *A. sericea* inhabiting the region of northern Bulgaria from whence the test insects for these studies came do not attack the two species implicated by Konow (1901) and Krishtal (1959), *F. vesca* and *F. viridis*, nor do they pose any threat to the cultivated strawberry, *F.* × *ananassa*. For the purpose of ensuring host fidelity in a weed BCC such as *A. sericea*, this is a satisfactory conclusion, as biological control agents are selected on a population-specific basis due to the need for absolute certainty in avoidance of non-target effects. The studies presented in this paper also confirm the use of *D. laciniatus* as food plant for *A. sericea*.

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# The cereal rust mite, *Abacarus hystrix*, cannot be used for biological control of quackgrass

**A. Skoracka[1] and B.G. Rector[2]**

*Summary*

Quackgrass, *Elymus repens* (L.) Gould, is a perennial grass spreading by vigorous underground rhizomes. Because of its capacity for rapid spread and persistence, it is considered as a common weed in many settings worldwide. The cereal rust mite, *Abacarus hystrix* (Nalepa), is a polyphagous, phytophagous mite attacking quackgrass and many other grasses including wheat. Its feeding causes leaf discoloration and inhibition of seed production. This mite can also transmit plant pathogens. Its role as a potential biological control agent for quackgrass control was considered since previous work had suggested that populations of this mite colonizing quackgrass may be specifically adapted to that host. The ability to colonize wheat by these quackgrass population should, however, be first excluded. The aim of this study was to estimate whether the cereal rust mite quackgrass population can colonize wheat. For this purpose, female mites from quackgrass were transferred and subsequently reared on quackgrass (control, $n$ = 132) and wheat ($n$ = 125). Colonization ability was assessed by comparing the mean oviposition rate, mean female survival and mean number of progeny on each host. Mites had similar success in the colonization of both quackgrass and wheat. The conclusion is that the quackgrass population of cereal rust mite is well adapted to wheat and thus cannot be considered as a potential agent against quackgrass.

***Keywords***: Acari, Eriophyidae, wheat, quackgrass.

## Introduction

Quackgrass, *Elymus repens* (L.) Gould [ex. *Agropyron repens* (L.) P. Beauv.], is a common and cosmopolitan species, occurring almost worldwide. It reproduces vegetatively by vigorous underground rhizomes, which, in turn, develop axillary buds that are capable of developing into new rhizomes and daughter shoots. Quackgrass is a highly aggressive, sod-forming, perennial grass native to Eurasia. Because of its invasiveness and persistence, it is considered as a serious weed of agronomic crops, turfgrass, landscapes, grasslands, gardens, lawns and nurseries in many parts of the world. In the USA, it is listed as a noxious and invasive weed introduced from Europe (Palmer and Sagar, 1963; Hultén and Fries, 1986).

Quackgrass is most effectively and commonly controlled by a combination of chemical and cultural methods. Herbicides for its control are available for most crops (e.g. Kells and Wanamarta, 1987; Ivany, 2002; Ivany and Sanderson, 2003). A few arthropods that live and feed on quackgrass are known, including *Hydraecia* spp. (Lepidoptera: Noctuidae) (Giebink *et al.*, 2000), *Delia coarctata* Fallen (Diptera: Anthomyiidae) (Marriot and Evans, 2003) and *Abacarus hystrix* (Nalepa) (Acari: Eriophyidae) (Frost and Ridland, 1996). However, no information on the biological control of this species was found in the literature.

The cereal rust mite (CRM), *A. hystrix* (Nalepa), is a phytophagous mite belonging to the family Eriophyidae. Eriophyid mites are often considered to be promising biological control agents for weeds because they debilitate their hosts by their feeding, they can transmit diseases to their hosts in certain cases and they tend to be highly host-specific (Rosenthal, 1996). Feeding on *A. hystrix* causes leaf discoloration and inhibition of host seed production. The mite is known to transmit plant pathogens, including ryegrass mosaic virus, a serious disease of temperate grasslands, and Agropyron

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mosaic virus, a minor disease of quackgrass (Oldfield and Proeseler, 1996). Thus, the mite is able to seriously damage its host plants. However, up to now, *A. hystrix* was not considered as a possible biological control agent due to its wide host range. The host range of the CRM includes records from at least 60 grass species, many of which are of economic importance (E. de Lillo and J. Amrine, 2007, personal communication). Among the hosts, the mite is dispersed passively by wind currents.

High host specificity is an extremely important characteristic in a biological control candidate. Above all, a weed biological control agent should not attack non-target plants, especially important crops. Despite many grass species having been recorded as hosts of *A. hystrix,* its role as an agent of quackgrass control was considered since previous work has shown that some CRM populations colonizing quackgrass may be specifically adapted to that host. Previous observations have shown that certain quackgrass and ryegrass populations of the CRM neither accept nor survive on each other's hosts (Skoracka and Kuczyński, 2006; Skoracka *et al.*, 2007). Moreover, the existence of reproductive barriers among these populations has been found (A. Skoracka, 2007, personal communication), suggesting that host populations of *A. hystrix* may represent a complex of species. If a quackgrass-associated population of the CRM is highly specific and adapted only to this host, it should be considered as a potential biological control agent of quackgrass control. Because quackgrass often grows near wheat, the potential for wheat colonization by a quackgrass-associated population of *A. hystrix* should be first excluded. Specifically, the aim of this study was to estimate whether the quackgrass-specific CRM population can colonize wheat.

## Methods and materials

Two grass species were used as host plants in this study: quackgrass and wheat *Triticum aestivum* L. Quackgrass rhizomes were collected in September 2006 from a study plot in Poznań, Poland (52°24.5′N, 16°53.0′E; elevation 87 m) and put in boxes with sandy soil. The plants were kept at room temperature and exposed to artificial light for 19 h per day. To protect the plants from infestation by mites, insects or fungi, the boxes were covered with nylon taffeta fastened to a wooden frame. When sufficiently grown, plants were used for the preparation of the stock mite colony and the experiment. A stock colony of mites was established with individuals collected from quackgrass from the same study plot and from two other study plots (52°25.0′N, 16°55.0′E, elevation 63 m, and 52°23.0′N, 16°52.0′E, elevation 79 m.) in October 2006. Females from field-collected plants were randomly selected and transferred to the un-infested plants in the laboratory. A detailed description of the stock colony preparation can be found elsewhere (Skoracka and Kuczyński, 2004). The colony was maintained in the laboratory (20°C, 40% ± 1 RH, 16–17:7–8 L/D) for 6 weeks. Afterwards, mites were used for the experiment.

Plants for the experimental were prepared as follows. Laboratory-grown quackgrass shoots were transplanted to pots, and wheat shoots were grown from seed. There was one grass shoot in each pot. Ten to fifteen females were transferred from the quackgrass stock colony to grasses grown in pots. Females were transferred under a stereomicroscope using an eyelash glued to a preparatory needle and put to the leaf of the grass. Two combinations were tested: (1) QQ – females from quackgrass transferred to quackgrass (control), $n = 134$ and (2) QW – females from quackgrass transferred to wheat, $n = 125$. Ten replicates were carried out for each combination. A replicate was defined as a single plant with 10 to15 mites transferred onto it. Each plot was covered on with nylon taffeta and maintained at 20°C, 40% ± 1 RH for 14 days. Afterwards, the plants were checked, and mites (number of experimental females and their progeny: eggs, larvae, nymphs and adults) were counted using a stereomicroscope.

Three components of colonization ability were measured for each treatment: (1) mean oviposition rate (total number of eggs oviposited by all females within the trial/total number of females tested within the trial), (2) mean female survival rate (total number of females survived within the trial/total number of females tested within the trial) and (3) mean number of progeny (eggs, larvae, nymphs and adults tallied separately) within each trial. Bootstrap (Efron and Tibshirani, 1993) was used to compute 95% confidence intervals (CI). Differences between means were tested using the criterion of CI overlapping (i.e. means were regarded as 'significantly different' when their CI did not overlap) and using a Hotelling's $T^2$-test and $t$-test.

## Results

No significant differences were shown between the ability to colonize quackgrass and wheat by the quackgrass-associated mites ($T^2$: $F_{3,16} = 0.49$, $p < 0.6946$). Specifically, there were no significant differences in mean oviposition rate ($t = 0.56$, $df = 18$, $p = 0.5865$), mean female survival ($t = 0.36$, $df = 18$, $p = 0.7215$) or mean number of progeny between mites developing on quackgrass and those developing on wheat (Table 1, Fig. 1).

**Table 1.** Mean oviposition rate and mean female survival with 95% confidence limits (in parentheses) of *Abacarus hystrix* that developed on quackgrass (QQ) and wheat (QW).

| Trials | Oviposition | Survival |
|---|---|---|
| QQ | 13.9 (11.7–-16.9) | 0.5 (0.4–0.7) |
| QW | 13.1 (11.8–-14.3) | 0.5 (0.3–0.6) |

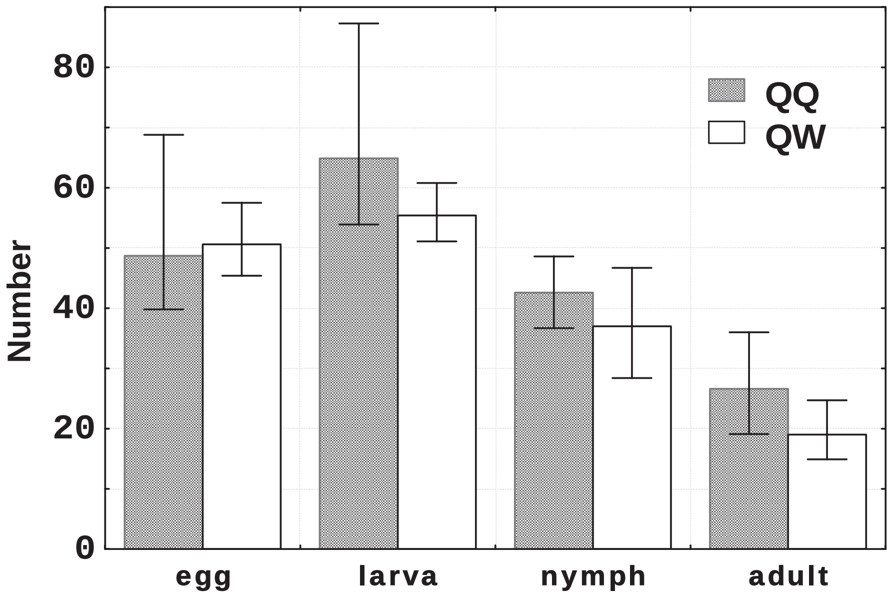


**Figure 1.** Mean number of progeny of *Abacarus hystrix* obtained on quackgrass (*QQ*) and wheat (*QW*). *Bars* represent 95% confident limits around means.

## Discussion

The idea of using eriophyoid mites for biological control of weeds has been of great interest since the 1970s. The characteristics that make eriophyids promising candidates for biological control are their frequent monophagy (or frequent specificity directed to one host), ability to suppress plant growth and reproduction, ability to destroy whole plant populations under favourable conditions, attack on all plant organs, particularly the inflorescences, and seed suppression (Rosenthal, 1996). A few eriophyid species have been used in biological control programs targeting weeds, e.g. *Aceria chondrillae* (Canestrini) to control *Chondrilla juncea* introduced to Australia and USA (Anders, 1983) and *Aceria malherbae* Nuzzaci to control *Convolvulus arvensis* in USA (Boldt and Sobhian, 1993). Many other species have been investigated and recommended for biological control, e.g. *Aceria tamaricis* (Trotter) (De Lillo and Sobhian, 1994), *Aceria centaureae* (Nalepa) (Sobhian *et al.*, 1989), *A. salsolae* De Lillo & Sobhian (Sobhian *et al.*, 1999), *Cecidophyes rouhollahi* Craemer (Sobhian *et al.*, 2004), *Epitrimerus taraxaci* Liro (Petanovic, 1990), *Phyllocoptes nevadensis* Roivainen (Littlefield and Sobhian, 2000) and *Floracarus perrepae* Khinicki et Boczek (Freeman *et al.*, 2005). Several European eriophyoids were suggested by Boczek and Petanovic (1996) for the control of weeds in North America in the genera *Cirsium*, *Lythrum*, *Convolvulus* and *Galium*. In addition, Boczek and Chyczewski (1977) found eriophyid mites in Poland associated with 56 weedy species.

Among the eriophyid mites causing notable damage to their grass hosts, *A. hystrix* is regarded as one of the most common and important. The mite has a great capacity for rapid population increase achieving high population densities (Skoracka and Kuczyński, 2004). At very high densities, the feeding of this mite causes plant wilting and delayed growth (Frost and Ridland, 1996). However, this study shows that the quackgrass-associated CRM had high fecundity, survival and development on wheat. It clearly appears that this quackgrass-associated population has great colonization ability and is well adapted to wheat and cannot be considered as a potential biological control agent against quackgrass.

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# Refining methods to improve pre-release risk assessment of prospective agents: the case of *Ceratapion basicorne*

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*Summary*

*Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae) is a univoltine weevil native to Eurasia whose larvae develop in root crowns of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). This insect was 'rejected' as a prospective biological control agent about 15 years ago after preliminary evaluation of its host plant specificity showed that it could develop on safflower (Clement *et al.*, 1989). However, it is known to attack very few plant species in the field and has never been reported from safflower. We conducted a series of no-choice, choice and field experiments to measure the risk that this insect would pose to non-target plants. Larval development occurred on nine species, including *Carthamus tinctorius* (safflower) and *Centaurea cyanus* (bachelor's button and cornflower). These host plants are within a small monophyletic clade within the Centaureinae. Three years of field studies conducted in eastern Turkey, at three sites with natural populations of the insect, demonstrated that the weevil does not damage safflower plants despite attack rates of 48–98% on *C. solstitialis*. A combination of taxonomic analyses and a hierarchy of host-specificity experiments were required to determine that this insect would be safe to introduce.

***Keywords***: host plant specificity, risk assessment, field experiments, phylogeny.

## Introduction

Determination of the host plant specificity of a prospective biological control agent plays a key role in the process of selecting and approving new biological control agents of weeds. Today, many consider host-specificity evaluation to be so routine that some professional journals refuse to publish such work. However, there still remains a great deal of 'art' to this work, which suggests room for improving the science. The goal of host-specificity evaluation is to predict the behaviour of agents after they are released into a new environment. To do this accurately is no small feat. Such predictions are usually based on the results of highly artificial laboratory experiments conducted under the constraints of working inside quarantine space. Furthermore, the tolerance of the public and regulatory agencies for risk of injury to non-target species has been decreasing. The combined effect is to reduce the probability of finding agents that can be approved for release. Can improving our methods help counter this trend?

There is a substantial literature reviewing methods of host-specificity evaluation, e.g. Clement and Cristofaro (1995), Withers *et al.* (1999), van Driesche (2000), Spafford Jacob and Briese (2005) and Sheppard *et al.* (2005). These reviews discuss many of the parameters that should be considered in the design of experiments. However, the choice of design should always reflect knowledge of the life history, ecology and behaviour of the agent. Thus, accurate testing requires customizing the experimental design for a particular agent on a particular target weed. A flow chart (Fig. 1) developed by Sheppard (1999) provides a starting point for choosing the most appropriate types of host-specificity experiments for a particular agent. However, given the multitude of experimental parameters that must be chosen for any experiment, these guidelines are only a beginning (Marohasy, 1998).

In this paper, we present an example of a prospective biological control agent that was once rejected based on preliminary experimental observations. However, because natural history observations suggested that the agent was more specific than what was indicated

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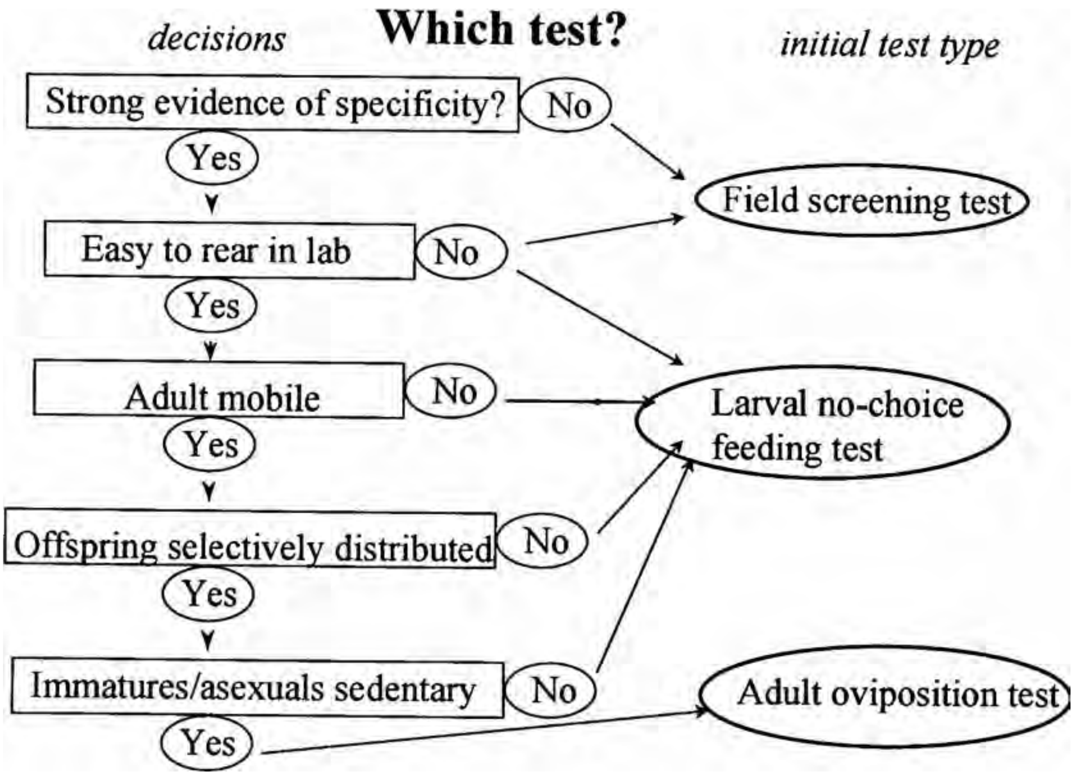


**Figure 1.** Decision tree for choosing general type of host specificity test (Sheppard, 1999). When adults of prospective agents are difficult to obtain or rear, the tendency may be to conduct larval transfer experiments. However, if the agent is naturally selective at the oviposition stage, then oviposition or field tests will give more realistic results.

by the experimental results, we conducted more thorough experiments, which have shown that the insect is indeed suitable for introduction. Nevertheless, preliminary studies are a critical early step in the process of focusing research efforts to efficiently develop new biological control agents. We will discuss what kinds of experiments are most likely to improve the reliability of such preliminary studies.

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae: Cardueae), is an herbaceous winter annual that is adapted to a Mediterranean climate: mild wet winters and dry hot summers (Keil and Turner, 1993; Roché and Roché, 2000). It is native to Eurasia and was introduced to the west coast of the USA over 100 years ago (Maddox, 1981). Seeds usually germinate in the autumn after the onset of winter precipitation. Rosettes grow during winter and spring, bolt in May to June and flower continually until frost or lack of moisture kills the plant. The plant has been the target of classical biological control in the USA since the late 1960s, but despite the introduction of six seed-head-attacking insects, it is not yet under control over most of its range (Turner *et al.*, 1995; Piper, 2001; Pitcairn *et al.*, 2004, 2006). This suggests the need for agents that attack vegetative parts of the plant and *Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae) was considered a likely prospect (Zwölfer, 1965; Rosenthal *et al.*, 1994).

*C. basicorne* is distributed throughout Europe and southwestern Asia, from Spain to Azerbaijan (Alonso-Zarazaga, 1990; Wanat, 1994). It commonly infests yellow starthistle in Turkey and Greece (Rosenthal *et al.*, 1994). This species has primarily been reared from yellow starthistle, but there are also reports of rearing it from *Centaurea cyanus* L. (bachelor's button and cornflower), *Centaurea depressa* Bieb. (which is very similar to *C. cyanus*), and in one case, *Cnicus benedictus* L. *C. benedictus* has recently been placed in the Jacea group of *Centaurea* (which includes yellow starthistle), based on phylogenetic analysis of DNA (Garcia-Jacas *et al.*, 2000). Thus, the insect has only been reared from a few species of plants in the Jacea and Cyanus groups, within the genus *Centaurea*. Adults have been found resting on plants only in the Cardueae tribe, which includes *Centaurea*.

*C. basicorne* adults emerge from hibernation in the early spring and feed on yellow starthistle leaves (Clement *et al.*, 1989; Smith and Drew, 2006). Females lay eggs in the leaves of rosettes from late March to early May (as observed in central Italy). Eggs hatch after about 10 days at room temperature, and first instar larvae mine in the leaf blade and down the petiole to the root crown. Larvae feed primarily in the root crown, complete development in about 2 months and pupate inside the plant. Adults emerge in June, feed on yellow

starthistle leaves for a few days and then disappear. They are thought to aestivate and hibernate in secluded places, and adults have been found under tree bark in July (Hayat *et al.*, 2002). Newly emerged females are in reproductive diapause, and although they mate, they are not able to lay eggs until completion of hibernation. In the spring, after feeding for 1–2 weeks, females lay a few eggs per day for 1–2 months before dying (Smith and Drew, 2006).

Clement *et al.* (1989) studied a population of *C. basicorne* in Italy. Because the species is univoltine and newly emerging adults do not oviposit, they used adults collected in the field on wild yellow starthistle plants. This greatly limited the number of adults that they could test (nine females). These adults were used in a few choice and no-choice feeding and oviposition experiments. Adults fed and oviposited on safflower under no-choice conditions but preferred yellow starthistle under choice conditions. Neonate larvae from these females were transferred to holes made in the central meristem of test plants. Larval development was observed on three non-target plants, including safflower. Because of these results, further evaluation of the insect was abandoned.

Considering that *C. basicorne* has never been reported as a pest of safflower and that field records indicate that it develops in only a few species of *Centaurea*, we decided to conduct a more thorough study of its host specificity.

# Methods

## No-choice oviposition

Individual mated females that had completed reproductive diapause were placed in a clear plastic tube (3.5 × 11 cm) mounted on an intact rosette leaf of a non-target plant species for 4 to 5 days (Smith, 2007). Each trial was preceded and followed by a positive control: placing the female with a cut yellow starthistle leaf for 2 to 3 days to determine if she could still oviposit. For each valid trial, we recorded adult feeding damage, oviposition and larval development. In general, we tested eight replicates per plant species in the tribe Cardueae and four in the more distantly related taxa. We doubled the number of replicates if there were any signs of larval development.

## Lab choice

An ovipositing female was placed inside a wooden sleeve box (73 × 43 × 43 cm; length, width and height) containing cut leaves of four to five species of test plants for 5 days. Each species was represented by a cluster of two cut leaves held in a vial of water with a foam stopper. Yellow starthistle leaves were included as a positive control in each trial. Adult feeding damage and oviposition were recorded. The number of valid replicates ranged from four to 18 for each of six non-target species tested.

## Field choice

We conducted experiments during 3 years (2002–2004) at three sites in eastern Turkey (Askale, Horasan, Çat) where *C. basicorne* was naturally abundant (Smith *et al.*, 2006). We tested two accessions of yellow starthistle: ‘US’ (seed collected in Davis, California) and ‘Turkey’ (seed collected at the three Turkish sites) and two commercial safflower, *C. tinctorius* L., varieties CW1221 (linoleic, CalWest) and S317 (oleic, SeedTec). All test plants were transplanted into the field sites as soon as *C. basicorne* feeding damage was observed on wild yellow starthistle plants. Plants were harvested as soon as *C. basicorne* pupae were observed in wild yellow starthistle plants and were either dissected or individually bagged to allow adults to emerge. Adult insects were identified by either Dr. Enzo Colonnelli (University of Rome ‘La Sapienza’, Italy) or Dr. Boris Korotyaev (Russian Academy of Sciences, St. Petersburg). Larvae were preserved in 99% ethanol for DNA extraction.

# Results and discussion

## No-choice oviposition

In no-choice oviposition tests, *C. basicorne* females oviposited on 94% of plant species in the subtribe Centaureinae (Smith, 2007), including safflower and the native species *Centaurea americana* Nutt. and *Centaurea rothrockii* Greenm. (results for Centaureinae are in Fig. 2). There was no larval damage to plants outside the tribe Cardueae. The highest occurrence of insect larval development was observed on *C. solstitialis* and *C. cyanus*, but there was significant development on *C. melitensis* L. (tocalote), *C. benedictus* (blessed thistle), safflower and *Crupina vulgaris* Cass. (common crupina). These results corroborate the previous observation that *C. basicorne* can develop in safflower (Clement *et al.*, 1989). However, neither safflower nor bachelor’s button appears to be normal hosts for *C. basicorne* because they do not form a rosette. Thus, when young larvae tunnel down a leaf on either of these plants, they cannot reach the root crown. Such larvae develop in the woody outer portion of the stem, not in the central pith. The relatively thin cortex provides a limited space for the insect and as the plant continues to grow, it sometimes crushes the pupa.

## Lab choice

In the sleeve-box choice experiment, adult feeding and oviposition was significantly greater on yellow starthistle than on any of the six other non-target

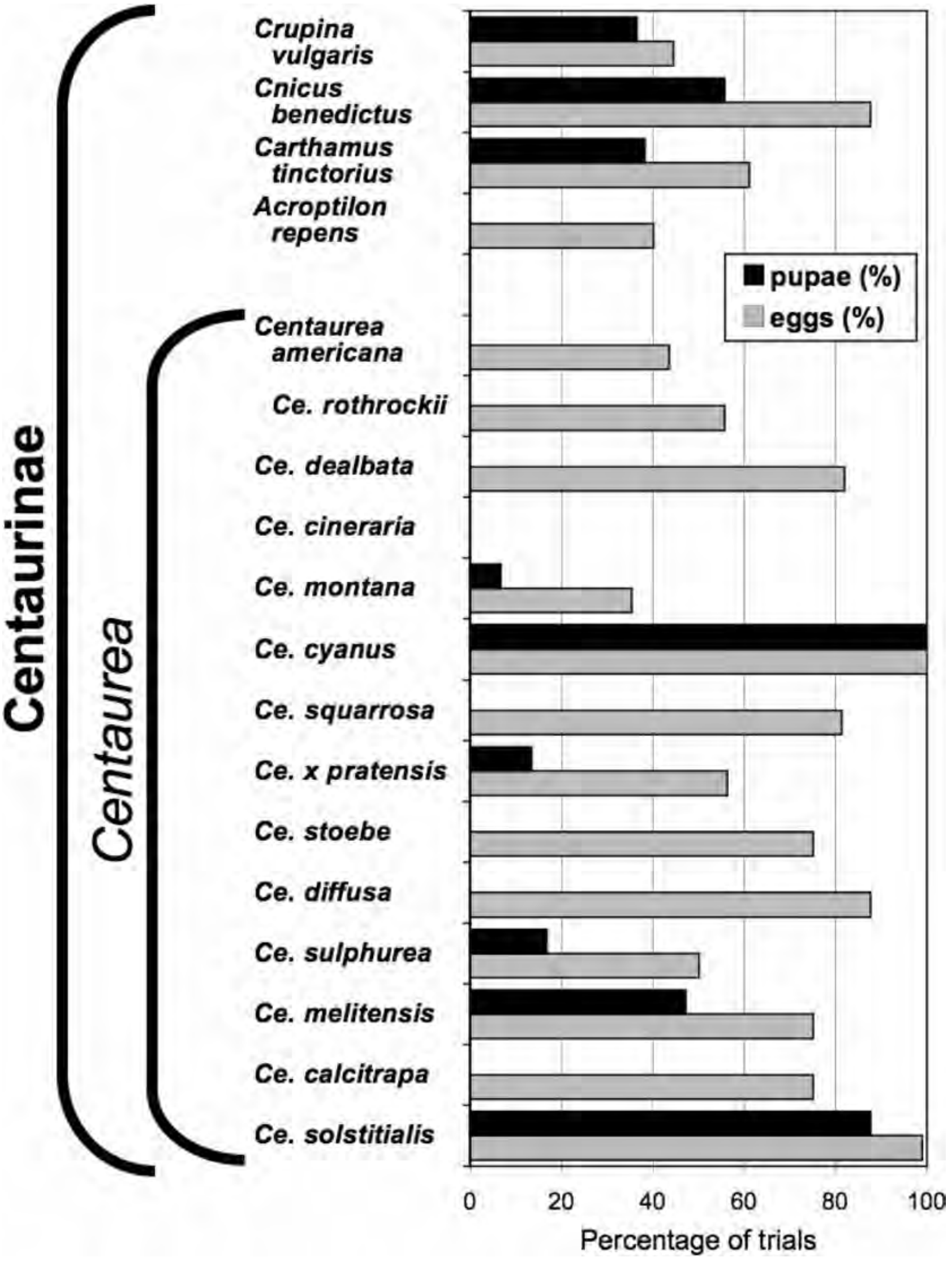


**Figure 2.** Proportion of trials in which a female oviposited on test plants ('eggs') and in which insects completed development to at least pupal stage ('pupae') in the no-choice host-specificity experiment. Individual females were held inside a plastic tube attached to the leaf of a non-target test plant for 5 days and on yellow starthistle for 2 to 3 days.

species tested. About 72% of eggs were deposited on yellow starthistle, 20% on bachelor's button, 5% on *C. melitensis*, 1% on *C. americana* and 1% on safflower (Fig. 3). These results indicate that *C. basicorne* females are more attracted to yellow starthistle than to bachelor's button or any of these other non-target test plants. However, bachelor's button and, to a lesser degree, safflower appear to be at risk of some attack, at least under these confined laboratory conditions.

## Field choice

The infestation of the yellow starthistle test plants was between 48% and 92% (US and Turkish plants

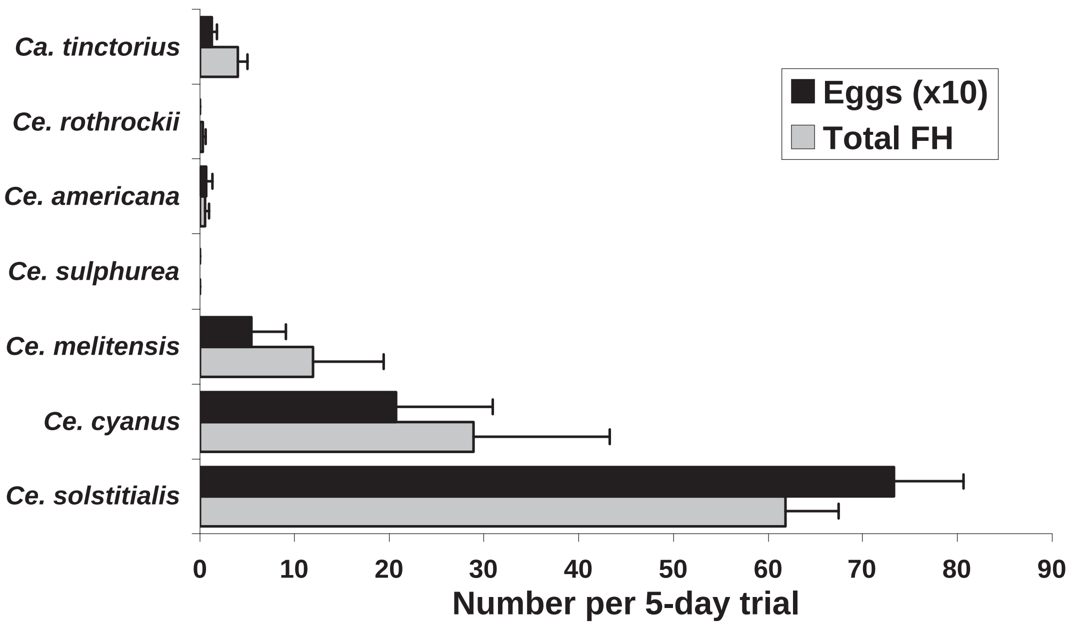


**Figure 3.** Oviposition and adult feeding by *Ceratapion basicorne* during choice oviposition experiments in sleeve boxes (one female for 5 days exposed to cut leaves of four to five plant species at a time, always including yellow starthistle). Number of eggs was multiplied by ten for visibility on the same scale; *FH* Number of adult feeding holes, each approximately 1 mm$^2$; error bars = SE.

**Table 1.** Infestation of root crowns or lower stems of test plants by apionid weevils (including larvae, pupae and adults) during 3 years at three field sites in eastern Turkey. *Ceratapion basicorne* was reared from yellow starthistle but not from safflower (Smith *et al.*, 2006).

| | Proportion of plants infested (%)[a] | | | | No. safflower plants sampled |
|---|---|---|---|---|---|
| | Test plant | | | | |
| | Yellow starthistle | | Safflower | | |
| Site | (US) | (Turkey) | Oleic | Linoleic | |
| **2002** | | | | | |
| Horasan | 83 b | 100 a | 0 c | 0 c | 45 |
| Çat | 28 b | 67 a | 0 c | 0 c | 38 |
| Askale | 59 b | 87 a | 19 c[b] | 16 c[c] | 40 |
| **2003** | | | | | |
| Çat | 37 a | 45 a | 0 b | 0 b | 57 |
| Askale | | 77 a | 8 b[d] | | 39 |
| **2004** | | | | | |
| Horasan | | 98 a | 0 b | | 250 |
| Askale | | 100 a | 26 b[e] | | 99 |

[a] Values followed by the same letter in the same row are not significantly different (chi-square test, $P < 0.01$)
[b] Adults identified: 4 *C. scalptum*, 1 *C. orientale*, 2 *C. onopordi*
[c] Adults identified: 2 *C. scalptum*
[d] Three unidentifiable adults
[e] Adults identified: 8 *C. scalptum*, 2 *C. orientale*

combined) at the three sites, indicating that there was a substantial infestation rate to challenge the safflower plants (Table 1). We reared 87, 145 and 297 individuals of *Ceratapion* from yellow starthistle at Horasan, Çat and Askale, respectively, but many were immature and others were in poor condition for taxonomic determination. All the adults that could be identified were *C. basicorne* (29, 30 and 92 from Horasan, Çat and Askale, respectively), except for two *C. orientale* (Gerstaecker). No safflower plants were infested by internal feeding insects at either Horasan or Çat. Thirty safflower plants were infested at Askale, but none of the insects reared from these plants were *C. basicorne*. We reared only *C. scalptum* (Mulsant and Rey), *C. orientale* and *C. onopordi* (Kirby) from these safflower plants. Subsequent identification of preserved larvae using molecular genetics has confirmed these results (Antonini *et al.*, 2008, this proceedings).

## Conclusions

Although *C. basicorne* can develop on safflower in laboratory experiments, we never found evidence of attack during 3 years of field studies. The results of our no-choice, choice and field experiments concur with current theory that no-choice experiments tend to overestimate risk to non-target plants under field conditions (physiological vs ecological host range). To avoid rejecting prospective agents that might actually be suitably host-specific, more emphasis should be placed on observations in the natural environment (i.e.

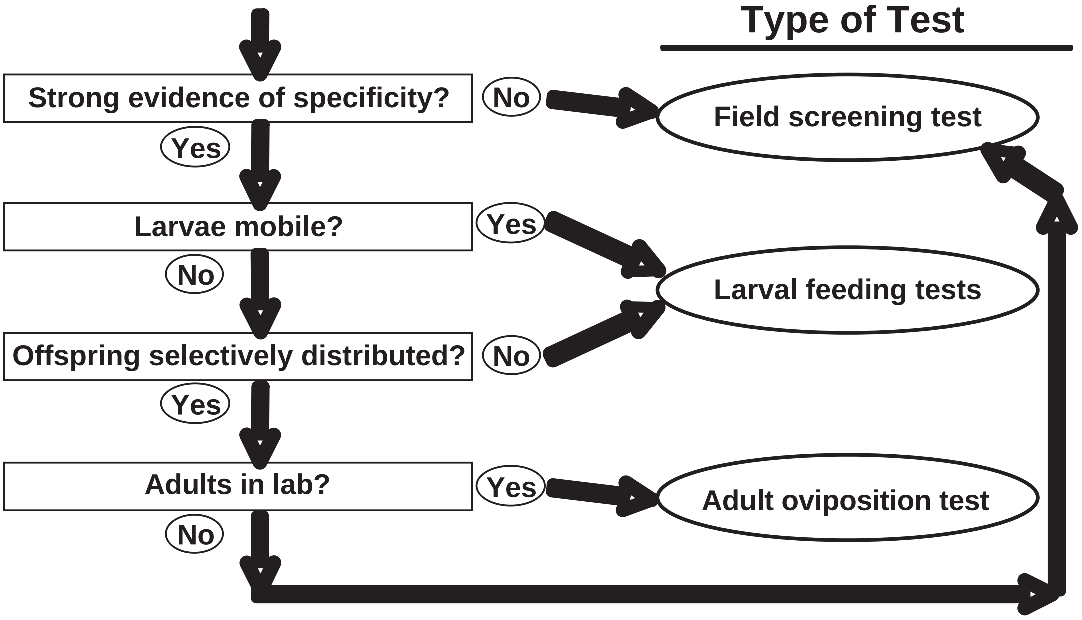


**Figure 4.** Revised decision tree for choosing the general type of host-specificity test. The emphasis is on what stages are capable of selecting the host. When suitable adults cannot be obtained by rearing or field collection, then field experiments should be used rather than larval transfer. Use of choice or no-choice experiments could be appropriate for any of these three types of tests.

natural history). This includes both examining non-target plants during foreign exploration and conducting relatively simple field experiments. Preliminary studies should focus on the most critical results for the particular agent. In this example, the critical issue was the attack on *C. americana*, *C. rothrockii* and safflower because they are the closest relatives to yellow starthistle deemed beneficial in North America. Preliminary experiments should focus on the developmental stage and conditions under which the agent chooses the target; in this case, females ovipositing on rosette leaves in early spring. Sheppard's (1999) flowchart provides a good guide, but with the caveat that for species in which the female chooses the host plant, adult oviposition should be tested regardless of whether the agent is easy to rear in the lab. The modified flowchart (Fig. 4) shifts the emphasis away from whether or not the agent is easy to rear in the laboratory towards which stage or stages choose the host plant. Conducting larval no-choice experiments for a species whose larvae are not capable of choosing their host plant is more likely to mistakenly eliminate it than if the adults were tested.

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# Host-specificity testing on *Leipothrix dipsacivagus* (Acari: Eriophyidae), a candidate for biological control of *Dipsacus* spp.

A. Stoeva,[1] B.G. Rector[2] and V. Harizanova[1]

*Summary*

*Leipothrix dipsacivagus* Petanovic & Rector is the first eriophyid mite recorded from hosts in the genus *Dipsacus* L. and is considered a potential candidate for biological control of invasive teasels (Dipsacaceae). Host-specificity testing on *L. dipsacivagus* (Acari: Eriophyidae) was carried out under insectary conditions for 4 months, from 19 April until the end of August, 2006. The laboratory colony of *L. dipsacivagus* was descended from mites collected on *Dipsacus laciniatus* L. in Klokotnitsa, Bulgaria. They were tested in choice and no-choice tests on *Dipsacus fullonum* L., *Knautia arvensis* L., *Cephalaria* sp. and *Scabiosa* sp. In the choice experiments, individual *D. laciniatus* plants infested with *L. dipsacivagus* were placed in cages with one to two plants of each test species. There were four replicates. Data were recorded at 10, 20, 30, 40, 60 and 90 days after infestation of test plants. After 10 days, only *D. laciniatus* was infested. After 20 days, mites were vagrant on all the plants except *Scabiosa*, and colonies were established on all *Dipsacus* plants. Some *Cephalaria* and *Scabiosa* plants had vagrant mites at days 20 and 30, respectively, but all of these were dead by days 30 and 40, respectively. By day 60, all *Knautia* plants were colonized, although these colonies later died. The mite successfully colonized only *D. laciniatus* and *D. fullonum* and temporarily colonized *K. arvensis*. In the no-choice tests, each of four plants of a given test species was infested with five mites/plant in a single cage. There were three replications (cages) for each test-plant species. Mites on plants other than *Dipsacus* spp. began to die after 10 days. Reproducing populations of *L. dipsacivagus* established only on *D. laciniatus* and *D. fullonum*.

***Keywords***: teasel, invasive plants, mites, Dipsacaceae.

## Introduction

*Leipothrix dipsacivagus* Petanovic & Rector is the first eriophyid mite recorded from hosts in the genus *Dipsacus* L. (Petanovic and Rector, 2007). This species was first collected in Serbia in 1999 but was misidentified (Petanovic, 2001). It was subsequently collected during surveys conducted in Serbia, Bulgaria and France in 2005, described as a new species (Petanovic and Rector, 2007), and is now a candidate for biological control of invasive teasels (*Dipsacus* spp., Dipsacaceae) in the USA. According to Petanovic and Rector (2007), the mite occurs on both the upper and lower leaf surfaces of *Dipsacus* spp. as a vagrant causing rust-like symptoms, wrinkles on the longitudinal folds of the leaves, 'witches broom' of the plant (i.e. reduced internode length and deformed leaves), stunting, delayed flowering and malformation of the flower heads. Analyses of symptomatic plant tissues for presence of microbial plant pathogens were negative (Petanovic and Rector, unpublished data). A study of the injuries caused by the mite (Pecinar et al., 2007) showed that the injury to the leaves is conspicuous at the morphological and physiological as well as the anatomical level.

Eriophyid mites are frequently considered to be promising candidates for weed biological control due to their high host specificity, rapid life cycle and severe damage to their host plants (Littlefield and Sobhian, 2000; Rancic and Petanovic, 2002; Sobhian et al., 2004). In one study, more than 80% of eriophyid

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species occurring on weeds were monophagous (Boczek and Petanovic, 1996), which is a key criterion for a successful weed biological control agent. A critical part of any biological control program is to test the host range of candidate agents to establish the level of risk posed to non-target plants and ensure that agent releases will not do more harm than good (Cullen and Briese, 2001).

The purpose of the present paper is to report on initial results concerning host-specificity testing of the new eriophyoid mite *L. dipsacivagus*, a potential candidate for biological control of *Dipsacus* spp., in the USA.

# Methods

## Origin and maintenance of test population

*L. dipsacivagus* individuals were collected from cutleaf teasel, *Dipsacus laciniatus* L., plants in a field near Klokotnitsa, Bulgaria (42°00.43′N, 25°27.41′E) and brought to the insectary of the Department of Entomology, Agricultural University in Plovdiv, Bulgaria. Species identification was made by R. Petanovic, Department of Entomology, Faculty of Agriculture, University of Belgrade, Serbia. The original mite colony was set up in August 2005 and maintained under insectary conditions on potted *D. laciniatus* plants.

## Test plants

Several closely related plant species from the family Dipsacaceae were chosen: *Dipsacus fullonum* L., *Knautia arvensis* L., *Scabiosa* sp. and *Cephalaria* sp. Plants of *D. laciniatus*, the original host of the colony, were used as a control. The test plants were grown in plastic pots 8 cm in diameter from field-collected seed. Plants were used in tests after they had formed their first two foliar leaves. Test plants were inspected to ensure that they were in healthy condition at the time of testing.

## Host-specificity testing

The host-specificity tests were conducted under laboratory conditions from 19 April until the end of August, 2006. Two types of cages made from clear, plastic panels with fine nylon mesh tops were used for the experiments: small (20 × 20 × 40 cm) and large (20 × 40 × 40 cm). During the tests, there was approximately 16 h of light per day in the insectary with temperatures of approximately 22°C during the day and 15° C at night. Relative humidity was 50% to 60% in the insectary and 70% to 80% within the plastic cages. Two different tests were designed for studying the host-specificity: choice tests and no-choice tests.

Choice test – Free migration was allowed from any infested *D. laciniatus* plant to other plants in the same cage. In each cage, one pot with an infested *D. laciniatus* plant was placed together with either three or seven pots of a test plant species, depending on the size of the cage. Plants were arranged in a randomized design within the test cages (four pots in the small and eight pots in the large cages). Pots were arranged such that the leaves of adjacent test plants within each cage were touching to facilitate migration of the mites between plants. For each plant species, four replications were made with each cage representing a replication. The plants were checked regularly under a stereomicroscope to monitor the migration of the mites. At 10, 20, 30, 40, 60 and 90 days, the number of test plants with mites present was recorded. The infestation was recorded as the number of infested plants in each cage and the percentage of infestation was calculated.

No-choice test – Potted plants of *D. fullonum*, *K. arvensis*, *Scabiosa* sp. and *Cephalaria* sp., were tested. *D. laciniatus* plants were tested in separate cages as a control. Five adult mites were transferred under a stereo microscope to the leaves of each test plant, using a fine brush. For each replicate, four plants of one test species were arranged in a small cage with three replications per species. The plants were checked under a stereomicroscope regularly. At 10, 20, 30, 40, 60 and 90 days, the infestation of test plants was recorded.

In both, the choice and no-choice tests migration, reproduction and feeding damage were recorded in addition to the presence of mites.

# Results and discussion

## Choice test

The initial results from the choice tests are presented in Table 1 and Fig. 1. By the tenth day after the beginning of the experiment, migration was observed only on *D. laciniatus* plants. After 20 days, migration was observed to all test species, except for *Scabiosa* sp. At that time, all plants in all control cages were 100% infested, while in the test cages, infestation varied from 0% on *Scabiosa* sp. to 50% on *D. fullonum* plants. After 30 days, 100% of the *D. fullonum* plants were infested, and after 60 days, 100% of the *K. arvensis* plants were infested. Migration to the other two species, *Cephalaria* sp. and *Scabiosa* sp., was observed at 20 and 30 days, respectively.

Soon after migrating, most of the mites on plants other than *Dipsacus* spp. died. By the 30th day on *Cephalaria* sp. and by the 40th day on *Scabiosa* sp., all the mites on these plants were dead. No further migration was observed to any of the test plants of these two species.

Besides the differences in the duration of dispersal, differences in the development of the mite depending on the host plant species were also noted. On the control (*D. laciniatus*), the mite began reproducing after 10 days, while on *D. fullonum* and *K. arvensis* plants, mites were reproducing after 20 days and 30 days, respectively. After 30 days, there were no differences in

**Table 1.** Results of host-specificity testing with *Leipothrix dipsacivagus* at lab conditions at the Agricultural University-Plovdiv in 2006.

| Test plant species | No of plants tested | No of infested plants (days after infestation) | | | | | | Feeding damage |
|---|---|---|---|---|---|---|---|---|
| | | 10 days | 20 days | 30 days | 40 days | 60 days | 90 days | |
| **Test 1. Choice test** | | | | | | | | |
| Dipsacaceae | | | | | | | | |
| *D. laciniatus* L. (control) | 20 | 17 | 20 | 20 | 20 | 20 | 20 | Yes |
| *Dipsacus fullonum* L. | 20 | 0 | 10 | 20 | 20 | 20 | 20 | Yes |
| *K. arvensis* L. | 20 | 0 | 6 | 12 | 17 | 20 | 20 | Yes |
| *Scabiosa* sp. | 20 | 0 | 0 | 7 | 13 | –[a] | –[a] | No |
| *Cephalaria* sp. | 20 | 0 | 7 | 13 | –[a] | –[a] | –[a] | No |
| **Test 2. No-choice test** | | | | | | | | |
| Dipsacaceae | | | | | | | | |
| *D. laciniatus* L. (control) | 12 | 12 | 12 | 12 | 12 | 12 | 12 | Yes |
| *Dipsacus fullonum* L. | 12 | 12 | 12 | 12 | 12 | 12 | 12 | Yes |
| *K. arvensis* L. | 12 | 9 (3) | 6 (6) | –[a] | –[a] | –[a] | –[a] | Yes |
| *Scabiosa* sp. | 12 | 8 (4) | –[a] | –[a] | –[a] | –[a] | –[a] | No |
| *Cephalaria* sp. | 12 | 12 | –[a] | –[a] | –[a] | –[a] | –[a] | |

Number in brackets shows the number of plants with dead mites.
[a] All mites are dead.

the activity of the populations on these two test-plant species and the control. The mite was reproducing and increasing its population density on *D. laciniatus*, *D. fullonum* and *K. arvensis* plants (with one exception) until the end of the experiment.

Feeding damage was observed only on plant species on which the mite successfully reproduced (i.e., *D. laciniatus*, *D. fullonum* and *K. arvensis*). For all three of these plant species, the oldest leaves of the rosettes wilted and became chlorotic and eventually withered. This feeding damage to rosette leaves under laboratory conditions was quite different from the damage to bolting and flowering *Dipsacus* spp. plants, observed by the authors in the field in 2004–2006, near the villages of Klokotnitsa (42°00.43′N, 25°27.41′E), Lozen (42°37.57′N, 23°30.34′E), Gorski Izvor (42°00.93′N, 25°26.14′E) and Dalbok Izvor, Bulgaria (42°11.34′N, 25°04.81′E; Stoeva et al., unpublished data) nor like that reported by Petanovic and Rector (2007). This could be due to physiological differences between rosettes and bolting or flowering plants or due to fundamental differences between field and insectary conditions.

On *Scabiosa* sp. and *Cephalaria* sp., although mites had migrated, they did not establish populations. Approximately 20 to 25 days after migration, the mobile forms had died. No eggs or immature stages were found on these plants. There was some evidence of feeding, although the leaves did not become chlorotic or dessicated.

An additional experiment was conducted for the test plant species on which development of mite populations had established. From the cages, housing choice tests involving *D. fullonum* and *K. arvensis* plants, the *D. laciniatus* plant, which had been the plant originally infested with mites in the choice-test cages, was removed. The cages were left with only the test plants (*D. fullonum* or *K. arvensis*), onto which mites had migrated and established populations. On the *D. fullonum* plants, mite populations continued to develop, whereas on *K. arvensis*, all mites died 30 days after removal of the *D. laciniatus* plants.

The development of the population on *K. arvensis* plants in the choice tests (when the test plants were touching the infested *D. laciniatus* plant) and the cessation of population development after removal of the *D. laciniatus* plant could be explained as a result of induction of a state of central nervous excitation (Marohasy, 1998) by the presence in the two *Dipsacus* spp. (but not in *K. arvensis*), of volatiles or other compounds that stimulate mite feeding or that are necessary for mite reproduction.

The results from the choice test showed that *L. dipsacivagus* migrates, feeds, reproduces and establishes sustained populations on *D. laciniatus* and *D. fullonum*, while it can feed temporarily on *K. arvensis* but cannot sustain itself on this host in the absence of *D. laciniatus*. Neither *Cephalaria* nor *Scabiosa* proved suitable as hosts to *L. dipsacivagus* in this experiement.

## No-choice test

Ten days after the beginning of the no-choice experiments, in which all plants were directly infested (five mites per plant), there were still live mites in all replications on all the tested plants (Table 1 and Fig. 1). Mites began to die after 10 days on some of the *Scabiosa* sp. and *Cephalaria* sp. plants and after 20 days on *K. arvensis* plants. By the 30th day, all the mites on all the plants of these species were dead. There was

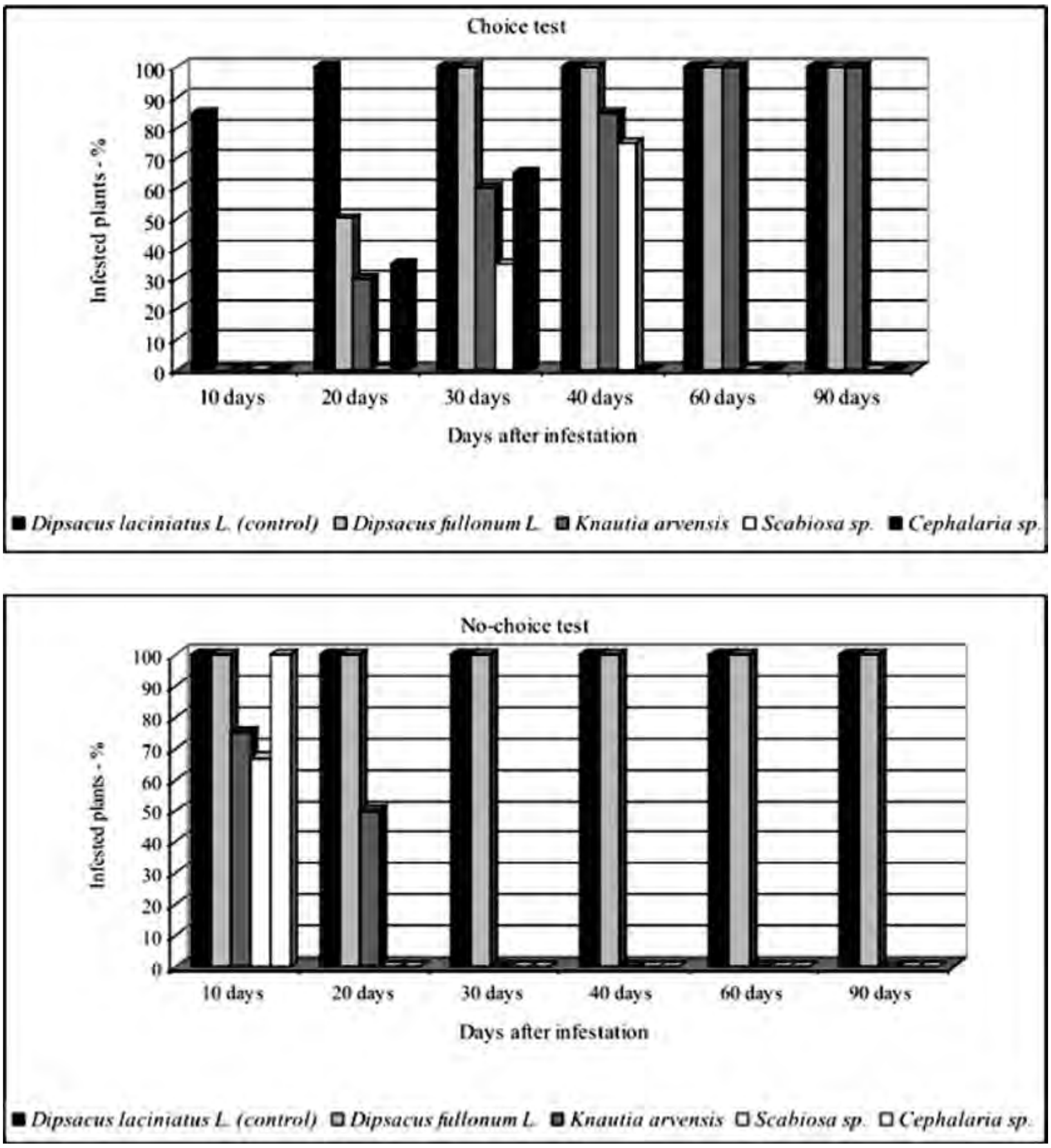


**Figure 1.** Infestation of *Leipothrix dipsacivagus* onto test plants in choice and no-choice tests under laboratory conditions.

no reproduction on these species, although there was some evidence of feeding. In contrast, on *D. laciniatus* and *D. fullonum* plants, there were eggs and immatures present on the tenth day and the population density was increasing. After 40 days, feeding damage was observed on these two species as described from the choice test.

The results from the no-choice test show that the mite *L. dipsacivagus* reproduces and establishes populations on *D. laciniatus* and *D. fullonum* plants, both of which are noxious weeds in the USA, and cannot colonize the confamilial species *K. arvensis*, *Scabiosa* sp. or *Cephalaria* sp. These preliminary results are promising, in that the effective host range of this mite appears to be restricted well within the family Dipsacaceae, if not within the genus *Dipsacus*. This would bode well for the prospects of this mite as a candidate for biological control of invasive teasels in the New World, as there are no native or economically important members of the family Dipsacaceae there.

Due to ambiguities in the results of choice tests of *L. dipsacivagus* on *K. arvensis*, further no-choice testing using larger initial infestations are under way. In addition, testing remains for the remainder of the host-specificity test list for this biological control candidate, which comprises approximately 40 species, mostly outside the Dipsacaceae, including rare and/or threatened native American plants in closely related families (e.g. Caprifoliaceae).

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# Impact of larval and adult feeding of *Psylliodes chalcomera* (Coleoptera: Chrysomelidae) on *Centaurea solstitialis* (yellow starthistle)

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*Summary*

The flea beetle, *Psylliodes chalcomera* (Illiger) (Coleoptera: Chrysomelidae), is a promising candidate biocontrol agent for yellow starthistle, *Centaurea solstitialis* L., a weed of primary importance in the western USA. Two sets of trials were performed to evaluate the impact of insect feeding at larval and adult stages. In the first experiment, larval impact was assessed by inoculating different numbers of neonate larvae on bolting yellow starthistle plants. The impact was evaluated on fresh/dry biomass, size of plant, numbers of seed heads, seed production and germinability. The second experiment was focussed on the evaluation of adult feeding behaviour, including possible gregarious aspects, which could result in a relative increase of damage on yellow starthistle. The larval transfer test showed a significant impact of larval feeding on yellow starthistle, although limited to seed production. *P. chalcomera* adult feeding was negatively influenced by the presence of other individuals on the same substrate. On the other hand, the individual feeding rates, especially for females, suggest a potential defoliation impact. Comparing the outcome of both experiments with the infestation rates observed in the field, *P. chalcomera* shows potential to damage yellow starthistle, although more studies are needed to assess the impact of such damage.

***Keywords***: impact assessment, flea beetle, feeding damage.

## Introduction

In 2001, a population of the flea beetle *Psylliodes chalcomera* (Illiger) (Coleoptera: Chrysomelidae) was observed developing on yellow starthistle, *Centaurea solstitialis* L. (Asteraceae: Cardueae), in the vicinity of the village of Volna, Krasnodar territory, southern Russia. This insect is known to attack *Carduus* species (Dunn and Rizza, 1976; Dunn and Campobasso, 1993), but it had not been previously observed feeding on species in the genus *Centaurea*. Subsequent behavioral and molecular genetic studies showed that the population associated with yellow starthistle was biologically different from populations associated with *Onopordum* sp. in Russia and *Carduus* sp. in Italy (De Biase *et al.*, 2003, 2004). Consequently, we are evaluating individuals from this population to determine if they would be suitable candidates for the biological control of yellow starthistle. An important part of this evaluation is to assess the potential impact that the insect can have on the target plant (Balciunas and Coombs, 2004).

Yellow starthistle is an important rangeland weed in the western USA, especially in regions with a mild moist winter and dry summer (Mediterranean climate; Maddox, 1981; Sheley *et al.*, 1999). It is a winter annual forb native to southern Europe and southwestern Asia. Seeds germinate in the late fall or early spring, rosettes develop until late spring and then plants bolt and flower until they senesce due to lack of moisture or freezing. *P. chalcomera* has one generation per year

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(Dunn and Rizza, 1976; Cristofaro *et al.*, 2004). Overwintering adults feed on yellow starthistle foliage in the early spring and oviposit on or near the plants. Larvae tunnel in the leaf midribs and young stems and exit the plant to pupate in the soil. Emerging adults feed briefly, mate and then aestivate and hibernate until the following spring.

# Methods

## Insects

Experiments on the impact of larval and adult feeding were carried out utilizing 65 adults of *P. chalcomera*, collected near Volna (45°07′36″N; 36°41′35″ E; altitude 16 m) on March 28 to 31, 2005. Based on our previous experience, adults collected at this time of year should be completing diapause and ready to begin feeding and ovipositing.

In the laboratory, the insects were placed in a 3-l glass beaker with crumpled paper and fresh yellow starthistle leaves at room temperature (18°C to 25°C) and with a 16:8 L/D cycle (natural and artificial light combined) for a week to allow complete reactivation after diapause. During this time mating was often observed.

Females and males were then separated. Males were kept in the same beaker described above, while females were individually placed in Petri dishes (25°C, 16:8 L/D cycle) with small bouquets of fresh yellow starthistle leaves with the aim to select ovipositing individuals. Laid eggs were collected daily, counted and placed in sterile Petri dishes over wet filter paper to allow hatching.

After 1 week, ovipositing females were put back to the common container with males, where the insects were allowed to feed on fresh yellow starthistle leaf bouquets replaced every other day.

## Larval transfer

The larval transfer test was carried out on early bolting, US biotype, greenhouse grown potted yellow starthistle plants. Two treatments and one negative control were set up transferring 10, 20 and 0 larvae per plant, respectively, with ten replicates each. Before the start of the experiment, plant height, root-crown diameter and number of internodes were recorded for each plant.

First-instar larvae that emerged from the eggs maintained on filter paper were transferred with a fine brush onto leaf axils, where larvae are known to enter the plant under natural conditions. To help the access of the larvae into the stem tissue, a small opening through the leaf tricomes was dug at each leaf axil with a sharp instrument. When enough neonate larvae were available, the transfers were done simultaneously on each replicate.

This first phase of the test was carried out in laboratory at 18°C to 26°C and 16:8 L/D cycle (natural and artificial light combined). Two weeks after the transfer of the first larva, each plant was enclosed in a 60- by 23-cm fine (1 mm) nylon mesh cage, supported by an inner aluminum frame and fastened around the outside of the pot with a 3-cm wide elastic band. The pots were then moved to a shade house outside the laboratory (6°C to 34°C min/max, 18.7°C mean temperature, 14:10 to 15:9 L/D conditions; April to June, 2005).

Forty days after transfer of the last larva, the cages were inspected to recover emerged adults. Such inspections were repeated every other day until the 60th day after the transfers, when all successfully developed adults were considered to have emerged. Cages were then removed and pot soil inspected for dead adults or pupae. At this time, corresponding to the full flowering stage, half the plants from each treatment ($n$ = 5) were harvested, carefully cleaned and weighed, and the number and stage of the flower buds, root-crown diameter and plant height were also recorded. Harvested plants were then dissected to estimate the mean number of galleries per plant, their position and average length. After dissection, the material from each plant was put together in paper bags, dried at 65°C for 72 h in a ventilated stove and weighed.

The remaining plants ($n$ = 5 for each treatment) were left undisturbed to allow the completion of the life cycle and seed production. When all flower heads were senescent, plants were harvested, measured, dissected and weighed following the above described methods. In addition, the seeds from ten mature flower buds sampled from each plant were collected, counted and separated into mature and immature. A germination test in Petri dishes was carried on sub-samples of mature and immature seeds from each plant.

## Adult feeding

The aim of this experiment was to assess the feeding impact of variable numbers of *P. chalcomera* adults on fresh cut yellow starthistle leaves by measuring the leaf area consumed per day. In addition, we wanted to investigate if the presence of one or more other individuals of the same or opposite sex on the same substrate would determine any significant increase or reduction of the amount of leaf tissue consumed in a specific time by an adult. The following combinations of males (M) and females (F) were tested: 1F, 2F, 5F, 1M, 2M, 5M, 1F + 1M, 2F + 2M and 1F + 1M. Each treatment was replicated ten times, except 5M (five replicates) and 5F (three replicates).

The trials were carried out in sterile 10-cm diameter Petri dishes in a climatic cabinet (25°C, 50% to 60% RH, 16:8 L/D cycle). The insects were allowed to feed on one single freshly cut yellow starthistle leaf, laid on a moist disk of filter paper for 24 h. The Petri dishes

also contained a moist sterile cotton plug (approximately 1 cm$^3$), a substrate to encourage oviposition.

Before each trial, leaves were carefully washed, rinsed and scanned into a TIF format digital image using a desktop flatbed scanner. At the end of each 24 h testing session, the leaves were again scanned. After each test, insects were placed back in a common container for at least one full day before insects were randomly selected for the next feeding trial. Eggs were collected daily, counted and placed in sterile Petri dishes over wet filter paper to allow hatching.

## Image analysis

Before performing image analysis, each image was edited with Photoshop LE v.5.0 (Adobe Systems, San Jose, CA) to colour the background and the eaten areas with two uniform tints (respectively, black and red). Such edits were achieved selecting background and eaten areas of the image with the magic wand tool, adjusting the tolerance level to fit their full width and finally coloring them with the paint bucket tool.

Next, the amount of leaf area eaten by adults of *P. chalcomera* was evaluated utilizing public domain software (Image v.4.0.3.2 beta for Windows, National Institute of Health, Bethesda, MD). Scanned leaf image dimensions were converted from pixels into square centimetres using the calibration function of the software and using a strip of scaled paper that was included in all scans as reference (O'Neal *et al.*, 2002).

Once calibrated, the red channel of each image was first inverted to negative and then converted to black

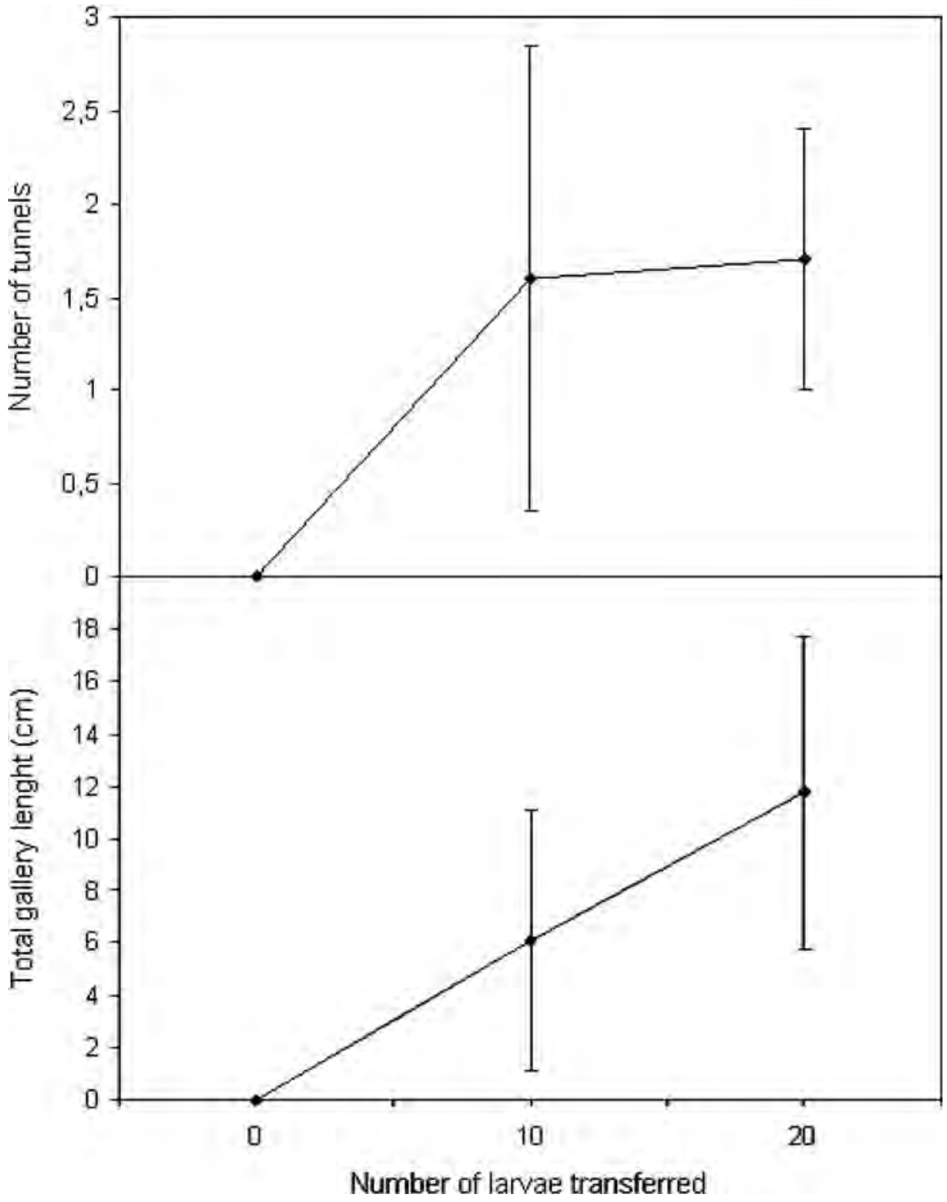


**Figure 1.** The length of galleries and the number of tunnels made by larvae of *Psylliodes chalcomera* when 0, 10 or 20 first-instar larvae were transferred onto yellow starthistle (mean ± 95% CI).

and white with the threshold function of the program. The threshold level was manually adjusted to fit the actual eaten area that was finally measured with the measure function of the software.

### Field infestation rates

*P. chalcomera* field infestation rates were assessed in May 2001 near Volna and Primorskii (45°15′08″ N; 36°52′17″E; elevation 5 m) on yellow starthistle, Italian thistle, *Carduus pycnocephalus* L., and nodding or musk thistle, *Carduus thoermerii* Weinm. In May and June 2003, two samples were repeated on the same population of yellow starthistle at Volna location only. At each sampling, 20 to 65 whole plants from dense patches were harvested, dissected and the numbers of larvae were recorded. In 2003, the crown diameter and height of the plants were also measured, and their fresh weight was evaluated.

### Statistical analysis

Data were analyzed using analysis of variance for classified effects or linear regression for quantitative effects. The relationship between total gallery length and number of seeds per capitulum was modeled by using least squares nonlinear regression with the Weibull equation using the quasi-Newton estimation method in the computer program Statistica (release 5.1, StatSoft Inc., Tulsa, OK).

## Results and discussion

### Larval transfer experiment

Establishment of larvae, as measured by the number of tunnels, was the same whether ten or 20 larvae were transferred to yellow starthistle plants; however, the total length of tunnelling increased with the number of larvae transferred (Fig. 1). Relatively few larvae were able to complete their development up to the adult stage: A total of five and seven adults were recovered, respectively, from 20- and 10-larvae treatments, which was only 2.5% and 7% survivorship, respectively. Although some larval mortality was expected, due to the experimental conditions that required ‘overcrowding’ larvae on the plants to obtain the maximum impact, an additional cause of mortality can probably be ascribed to the dispersal behaviour of newly transferred larvae. We observed some larvae moving away from the intended insertion point on the plant and they fell from the plant and possibly died.

Larval damage (total tunnel length) did not affect wet weight, dry weight, number of buds, number of mature flowers, number of senescent flowers or total number of capitula. This is probably because (1) these plant characters are influenced by plant growth that occurred before the insect damage occurred, (2) the plants were able to compensate for the damage or (3) the damage was too small to affect the plant. However, feeding by *P. chalcomera* larvae significantly reduced the number of seeds produced per capitulum (whether or not dry

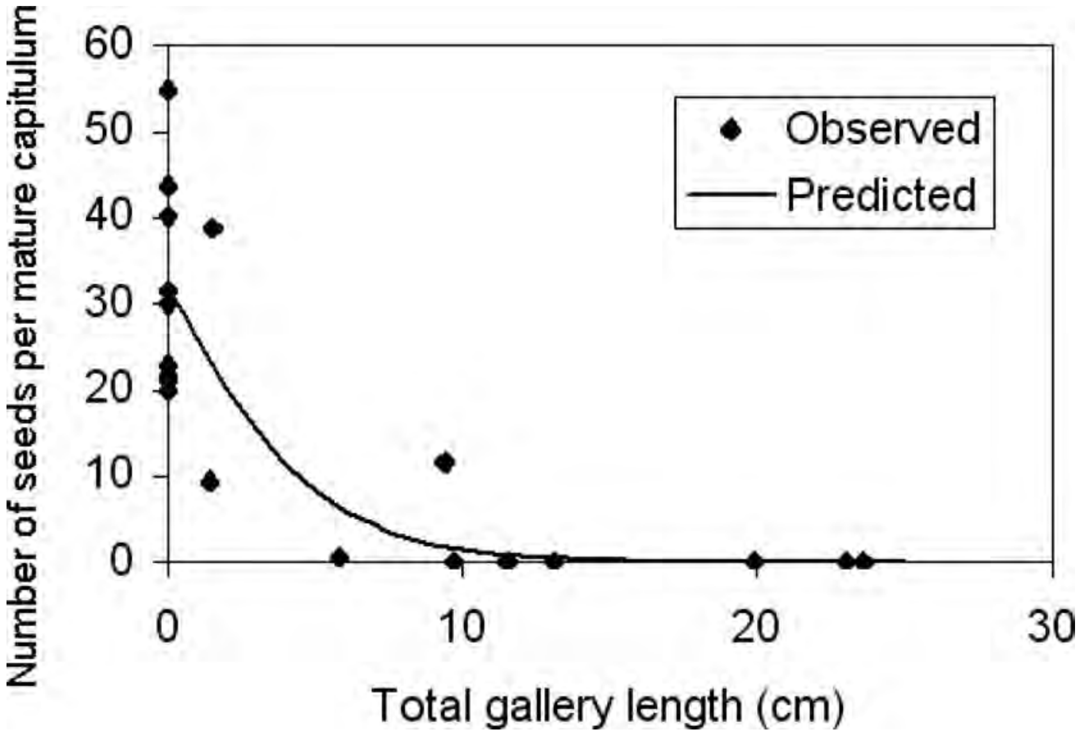


**Figure 2.** The relationship between total gallery length, made by *Psylliodes chalcomera* tunneling in yellow starthistle, which increased with greater numbers of larvae, and the number of seeds per mature capitulum.

weight was included as a covariate). The data were fit by the Weibull equation ($Y = c \times \exp(-((X/a)^b))$, where $c = 31.786 \pm 3.548$ (SE), $a = 3.839 \pm 2.465$, $b = 1.188 \pm 1.175$, $R = 0.830$). The equation predicts that 8 cm of larval tunnelling is able to reduce seed production by 90% and 14 cm by 99% (Fig. 2). This suggests that the larval damage reduced the plant's ability to provide nutrients to developing seeds. The number of capitula per dry weight of plant decreased slightly with increasing length of larval tunnelling (slope = -0.098 ± 0.035 SE; $F_{(1, 27)} = 8.0$; $P < 0.009$), suggesting that larval damage decreased resources available to develop capitula.

## Adult feeding experiment

Females feed more than males [area eaten (1F vs 1M): females = 0.416 ± 0.058 (SE), males = 0.027 + 0.007 $cm^2$, $F_{(1, 18)} = 43.7$, $P < 0.0001$; and feeding scars per individual: females = 11.700 + 1.627 (SE), males = 2.300 + 0.517, $F_{(1, 18)} = 30.33$, $P < 0.0001$] (Fig. 3). Feeding per insect was not affected by crowding at the observed levels for either males or females, when each sex was analyzed alone (slope of linear regression for area eaten or number of feeding scars per individual, $P > 0.05$). However, there was a significant interaction between sex and number of insects for both response variables that was related to a tendency of feeding by females to decrease with crowding while feeding by males remained constant.

## Field infestation rates

The infestation rate of yellow starthistle plants observed in southern Russia ranged from 36% to 90% of yellow starthistle plants sampled in May or June in 2001 and 2003 (Table 1). The number of larvae per infested plant ranged from 2.3 to 6.9. No larvae were observed in nearby plants of *C. pycnocephalus* at the Volna site, but 20% of *C. thoermeri* were infested at the Primorskyi site (Fig. 4). Although *C. thoermeri* plants were larger than yellow starthistle, the number of larvae per infested plant was lower. These results suggest that either *C. thoermeri* is a more suitable host than C. *pycnocephalus* or that the insect populations on

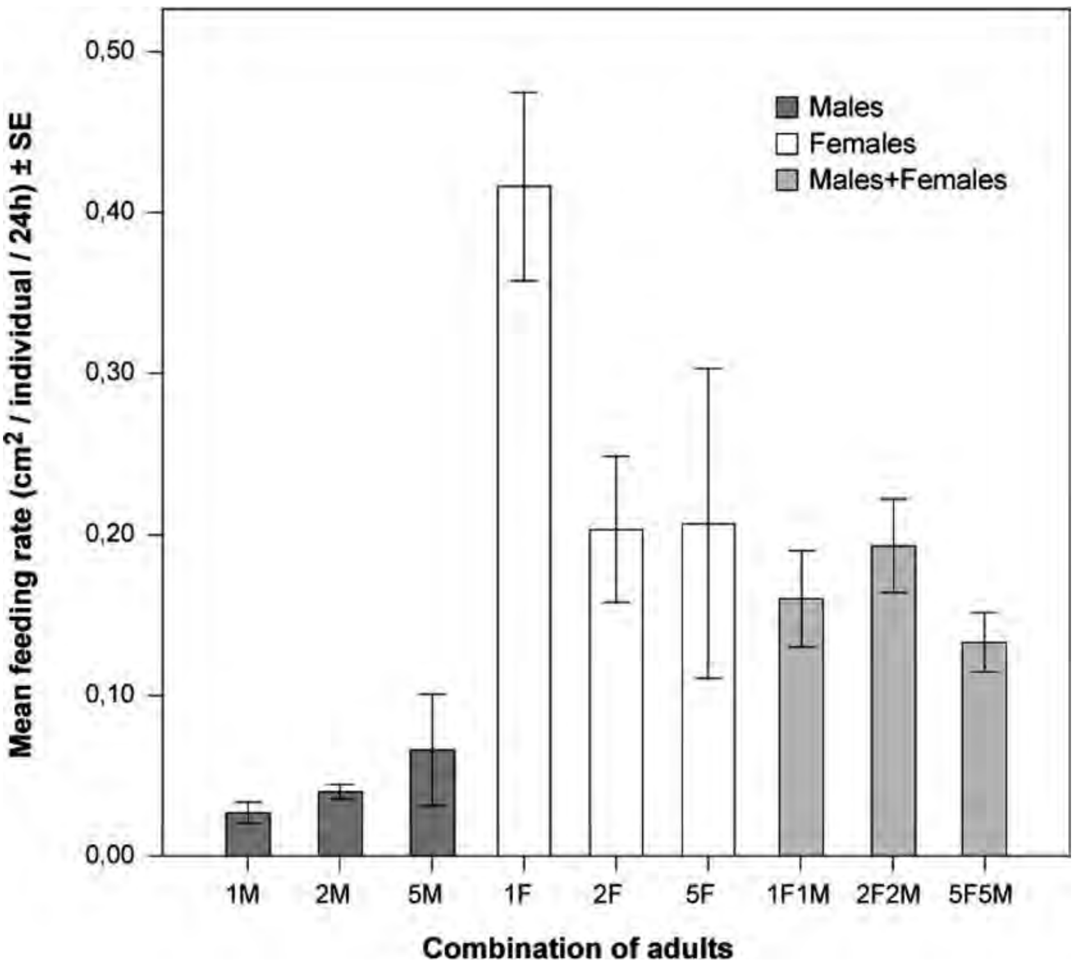


**Figure 3.** The effect of crowding different numbers of adult males and females on mean feeding rates per individual of *Psylliodes chalcomera* on leaves of yellow starthistle during 24 h (*1M* one male, *1F* one female, *2F2M* two females with two males etc.)

**Table 1.** The infestation rate and the number of larvae of *P. chalcomera* per infested plant species observed at two sites in southern Russia (±SE).

| Location | Date | Host plant | Infestation rate | No. larvae/ infested plant | No. plants sampled |
|---|---|---|---|---|---|
| Volna | 10 May 01 | *C. solstitialis* | 38% | 2.33 ± 0.44 | 40 |
| Volna | 10 May 01 | *Carduus pycnocephalus* | 0% | 0% | 20 |
| Primorski | 11 May 01 | *C. solstitialis* | 36% | 2.71 ± 0.48 | 50 |
| Primorski | 11 May 01 | *Carduus thoermeri* | 20% | 1.20 ± 0.20 | 50 |
| Volna | 19 May 03 | *C. solstitialis* | 63% | 4.34 ± 0.54 | 65 |
| Volna | 16 June 03 | *C. solstitialis* | 90% | 6.91 ± 0.63 | 48 |

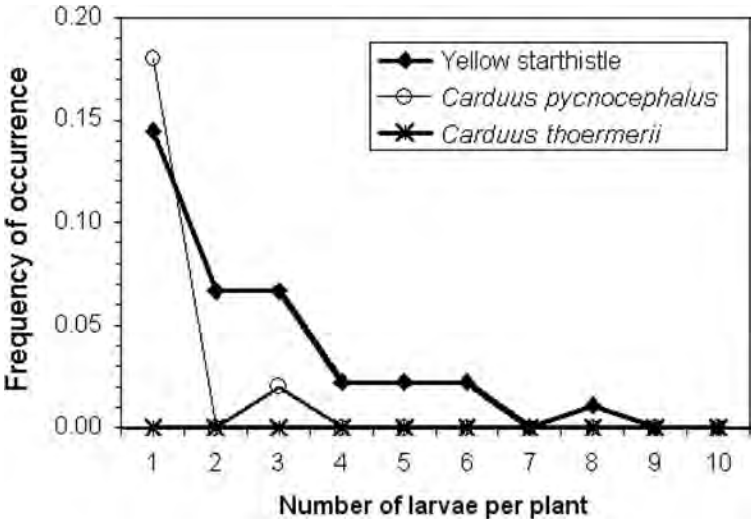


**Figure 4.** The frequency distribution of number of larvae of *Psylliodes chalcomera* attacking some Cardueae plants in the field at sites in southern Russia.

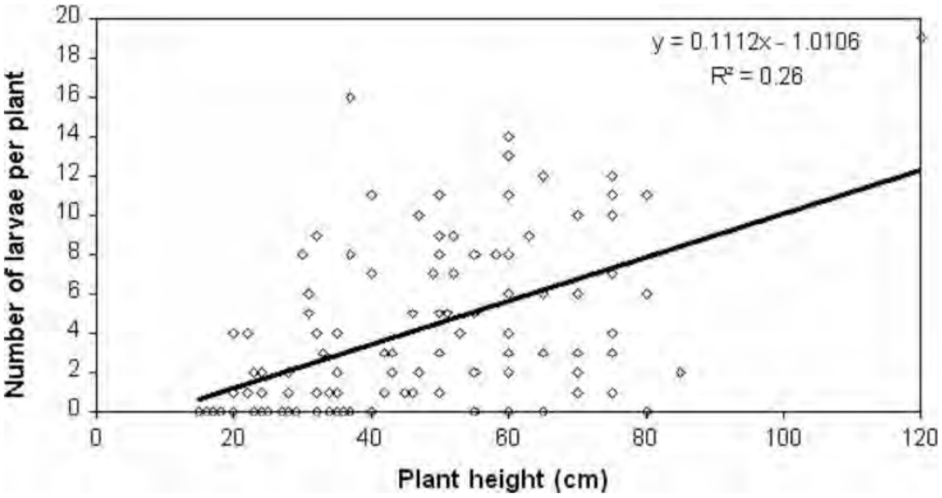


**Figure 5.** The relationship between number of larvae of *Psylliodes chalcomera* per plant of yellow starthistle and plant height in the field at sites in southern Russia (sampled in May and June 2003).

these two plants may be genetically different. The latter theory is supported by observations in laboratory host plant specificity experiments and analysis of molecular genetics, which indicate at least three host-associated genetically isolated populations of this morphological species (Cristofaro *et al.*, 2004; De Biase *et al.*, 2003, 2004).

The number of larvae per plant increased with plant size, as represented by either plant height or root diameter (Fig. 5). Although plants were taller on the June sample than in May [64.2 ± 2.0 (SE) vs 34.1 ± 1.4 cm], sample date did not significantly affect the relationship between the number of larvae and plant height. The best-fitting regression equation was Y = –1.01 (±0.90 SE) + 0.111(±0.018) × *X*, where *X* = plant height (cm) ($F_{1,111}$ = 39.00, *P* = 0.0001), but the regression only explained about a quarter of the variation. Because larvae were probably too small to significantly affect plant height at the time of sampling, the results suggest that larger plants either attract higher rates of infestation or that they are able to support more larvae (which may be cannibalistic). If this tendency of finding more larvae on larger plants holds true, it is an attractive property for a prospective biological control agent because higher numbers are probably necessary to impact larger plants.

## Conclusions

Feeding damage caused by larvae at the levels that we observed in the laboratory, where they produced one to five galleries and zero to two adults per plant, was not sufficient to reduce plant size of yellow starthistle plants. However, larval damage greatly reduced the number of seeds per capitulum and the number of capitula per plant biomass, which is very interesting damage to inflict on an annual plant. Transfer of about 12 larvae corresponded to 8 cm of tunnelling, which cause 90% reduction of seed production. In Russia, the number of larvae observed in the field was much lower (generally less than four larvae); however, it is not known whether the studied population is limited by natural enemies. If that was the case, then impact of this insect could be substantial when established in a region lacking these natural enemies (Torchin *et al.*, 2003). Further studies are ongoing to improve the survivorship of transferred larvae in laboratory to evaluate the larval feeding impact with infestation rates similar as the ones recorded in natural conditions.

Although adult females fed more (11.0 scars, 0.41 $cm^2$ per day) than males (2.3 scars, 0.03 $cm^2$) and, in the field, feeding is likely to occur during 2 to 3 months in the spring, unless they have gregarious behaviour or preferentially attack young plants, this level of damage is not likely to significantly affect plant growth. Our crowding experiment showed that the adults feed less when crowded, and they generally have not been observed to aggregate on plants in the field. However, preference for or impact on young rosettes has not been studied.

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# *Syphraea uberabensis* (Coleoptera: Chrysomelidae) potential agent for biological control of *Tibouchina herbacea* (Melastomataceae) in the archipelago of Hawaii, USA

**C. Wikler[1] and P.G. Souza[2]**

*Summary*

Biological invasions are one of the major threats to Hawaii's biodiversity. Herbacious glory tree, *Tibouchina herbacea* Cogn. (Melastomataceae), native to America, is regarded as one of the harmful plant species due to its fast growth, small wind dispersed seed and the absence of natural enemies. Since 1998, potential agents have been studied in Brazil for a classical biological control. This paper presents results from host range and impact tests conducted under field and laboratory conditions for *Syphraea uberabensis* Bechyné (Coleoptera: Chrysomelidae), which is indicated as having great potential as a control agent for *T. herbacea*. A detailed description of the adults of this insect has not been published, and we describe it in this paper.

From a group of 20 species of plants in ten families investigated, *S. uberabensis* fed only on the two species of *Tibouchina* demonstrating that *Tibouchina* supplies the physiological and biological needs of this insect. *S. uberabensis* completes its life cycle on the leaves of *T. herbacea* and does not attack other plant parts. During summer months, the life cycle is completed in approximately 35 days, lengthening to 80 days in the cooler months. The main impact to the plant was caused by the third-instar larvae and adults, and the damage can kill plants in less than 2 weeks. In laboratory conditions, 25% of leaf damage caused leaf death and leaf drop. A batch of *S. uberabensis* was sent to the Quarantine Service of USDA in Hawaii in 2005 where further host-specificity tests are being conducted.

***Keywords***: host range, impact tests, *Tibouchina*, Hawaii.

## Introduction

Herbaceous glory tree, *Tibouchina herbacea* Cogn., native to southeast Brazil, Uruguay, Paraguay and Argentina, has become a particularly troublesome species in the Hawaiian Archipelago (Almasi, 2000). Its vigorous spread by tiny seeds and sprouts is beyond conventional control techniques, and it has been the target of extensive field research in Brazil since early exploratory work was conducted in 1994 by Burkhart (1994). Since 1998, a biological control research program targeting this aggressive and tenacious weed has been underway in southern Brazil, and potential biological agents are being evaluated. Among them, the flea beetle *Syphraea uberabensis* Bechyné (1955) is the highest ranked potential candidate according to the field and laboratory studies conducted in Irati, Brazil (Mueller and Wikler, 2001). Though some species of *Syphraea* are known to feed on the roots of the target weed, *S. uberabensis* has only been observed feeding on leaves.

The genus *Syphraea* is described by Baly (1876) as oval, compact, small black or blue-black flea beetles. *S. uberabensis* are 3–4 mm in length and have a dark blue color. The antennas have robust articles from the base to the apex compared with the anterior tibia; the elytra have simple and very fine punctuations (Bechyné, 1955). This paper provides further description of *S. uberabensis*, its biology and its impact on *T. herbacea* and results of host specificity tests.

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## Methods and materials

Seedlings of *T. herbacea* from different provenances were reared for the experiments in greenhouses at the Irati campus. Cultures of *S. uberabensis* were obtained from *T. herbacea* and *Tibouchina cerastifolia* Cogn. plants that occurred naturally at the Irati Campus and Mananciais da Serra, Paraná State and from the locality of Nova Petrópolis, Rio Grande do Sul State.

Insect rearing, biological observations and experiments were conducted under laboratory conditions in a controlled temperature room ranging from 29 ± 2C° day to 22 ± 2C° night, under artificial light banks on a 12-h photo phase. Morphological descriptions were made of insects that were reared under these conditions.

Biological observations and host range experiments were conducted in Petri dish (10-cm diameter) and in plastic bottles of 200 ml, with wet filter paper at the bottom. Leaves of *Tibouchina* species were used to feed individual larva and adult *Syphraea*. The larvae and adults were examined every second day, and host plant leaves were substituted daily to reduce the incidence of diseases and to provide fresh food.

In the plastic bottles, seedlings of both species of *Tibouchina* plants (20 to 30 cm tall) were placed in about 10 cm of sterilized soil collected from the same sites as the plants to provide the whole plants to the insects. Frass was not removed, and no additional plant material was provided.

Based on the phylogenetic system of Cronquist (1981), 20 plant species that were closely related to the *T. herbacea* and occurred in similar habitats were selected for host-specificity tests (Hight *et al.*, 2003). Plants from the families Alzateaceae, Crypteroniaceae, Oliniaceae, Penaeaceae, Punicaceae, Rhynchocalycaceae, Sonneratiaceae and Trapaceae were not included in the tests due their absence in the study region.

For the no-choice tests, neonate's larvae reared at the laboratory and adults collected in the Irati Campus field were placed in Petri dish containing moist filter paper and leaves from the test plant. Four replicates of each test plant were used. After all larvae and adults had died in each dish, the leaves were observed for feeding indicated by scraping of leaf epidermal cells and for larval frass. Leaf area and consumed areas were measured by tracing the leaf outlines and the eaten areas on millimetre paper and counting the areas damaged or missing. Counts of the eggs and initial instars were conducted using a Wild stereoscopic microscope at 10× and 40×. Data were analyzed by conventional statistics using Microsoft® Excel.

## Results

### Description of adults of *S. uberabensis*

**Body:** Elongated, slightly broader posteriorly; robust legs; thorax, abdomen, legs and antennae relatively covered with fine short hairs; coloration deep metallic blue, 2.8 ± 0.10 mm long and 1.5 ± 0.03 mm wide.

**Head:** Top of the head with strong depressed area before frontal calli; punctuation behind frontal calli, longitudinal carina narrow, elevated, eyes small and oblong, entire; antennae more or less thickened.

**Thorax:** Pronotal punctures small, larger antebasal sulcus but not deeply impressed; lateral margins of pronotum narrow; prosternal narrow with dense and long setation, extending a little beyond posterior margin of procoxae; metasternum densely covered with fine soft hairs.

**Elytra:** Large punctures densely distributed and deeply impressed toward the base getting a little apex; elytra not smooth; few short setae on posterior margin of elytra.

### Biology of *S. uberabensis*

Most information presented in this paper is from the laboratory experiments. When possible, we have provided ranges for summer and winter field conditions. Mating occurred predominantly on the under surface of the leaf toward the apex of the leaf, although, in both laboratory and field situations, we occasionally observed mating on the upper surface. Mating occurred during the night, early hours of daylight and evening, but on rainy days and periods of high humidity, it occurred throughout the day. Mating was effected by the female partially opening their elytra not only to facilitate the appropriate juxtaposition of the male but also allowing for a quick disassociation in case of predation. We observed that mating frequency was reduced when food was scarce, especially in autumn and winter when the plants were in decline or reduced to their overwintering, short shoots.

The eggs were white and elliptical, measuring 0.58 ± 0.01mm by 0.25 ± 0.01mm. Oviposition was observed only in the laboratory because it occurred principally during the cooler hours of darkness and to a lesser extent the early morning or late afternoon. The female pushed the leaf hairs apart with the hind legs and deposited eggs among the hairs on both surfaces of the leaf and on the stem. On *T. herbacea*, the eggs were interspersed between the abundant leaf hairs, but on *T. cerastifolia*, the eggs were cemented on the leaf cuticle. Eggs were normally laid singly, although rarely two or three eggs were found together. It was not known if these eggs were laid at the same time or that females later visited the same site (Wikler and Souza, 2005). Egg laying began 8 ±3 days after copulation in the lab and approximately 12 days after copulation in the field in autumn. The maximum number of eggs oviposited during a single session of 3 h in the lab was 42. The average number of eggs laid in the Petri dishes was 44 ±0.5 per week, although in the first eight weeks, it is higher than 65 eggs per couple and during this period most individuals died ($n$ = 57 mating pairs; Fig. 1). Eggs took between 12 and 21 days to hatch, and the emerging larvae were active and mobile.

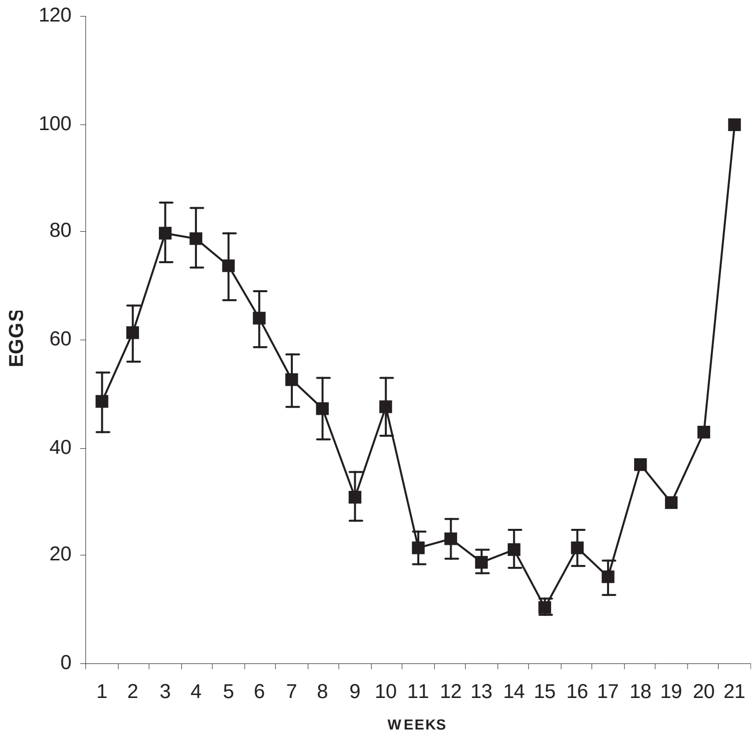


**Figure 1.** Weekly average number of eggs oviposited by mated females of *Syphraea uberabensis* (Coleopera: Chrysomelidae) under laboratory conditions in a controlled temperature room.

First-instar larvae were 1.95 ± 0.02 mm long, whitish and becoming yellowish on the second day. They have three to four segments, and the head is not well defined. This stage was brief lasting an average of 1.87 days. Feeding damage by these young larvae was almost imperceptible. The second-instar larva was light yellowish color, with five to seven segments and a well defined head, although it was often difficult to distinguish and was best observed from the underside. There was a small posterior protuberance. Initially, the larvae were 3 mm long, growing to 5 mm before ecdysis. This stage lasted from 6 to 12 days. Feeding damage was insignificant and did not exceed 1 $cm^2$ per week. Third-instar larvae were dark yellow, with seven segments, a well-defined head and a large posterior protuberance. It was 6 mm long. Feeding damage was more than 1.27 $cm^2$ per week, producing the most significant damage to the plant, greater even than the adult. This stage lasted 12 to 25 days, the first half feeding and the second half entering the soil in prepare for pupation. The pupae were initially the same color as the third-instar larva, darkening only a few days before eclosion.

Adults emerged as soon as 10 days after pupation, although in the field in winter it took at least 30 days. In the laboratory, of 48 insects, only 50% survived 7 weeks, 25% for 11 weeks and all had died by the end of the 21st week

Mortality during development of this insect was high; 65.7 ± 17.2% of the eggs survived to first instar, 55.8% ± 21.4 % to the second instar, 51.2% ± 20.0% to third instar and 7.6 ± 7.7% of eggs survived to adulthood. In only one collect in Rio Grande do Sul, two generalist Hemipterans (not yet identified) were found attacking the adult insects in the field. Fungi also attacked the larvae and pupae during periods of high humidity in the laboratory.

Feeding occurred without preference on both younger and older leaves. Visual observations of leaf consumption by adults and especially third-instar larvae was extensive and resulted in reduced plant viability, lack of flower maturity and reduced seed production (C. Wikler and P. Souza, unpublished data).

## No-choice tests

In no-choice tests, *S. uberabensis* laid eggs on and larvae and adults fed on plants in the genus *Tibouchina* but not on any other test plant (Table 1). Additional feeding trials conducted in the laboratory showed that larvae completely defoliated *T. herbacea* in no-choice tests and were able to complete development to adults on this species. In choice tests, larval preferences for *T. herbacea* and *T. cerastifolia* were equal.

## Effects on *T. herbacea* and *T. cerastifolia*

In the experiments of biological impact, adults and larvae of *S. uberabensis* demonstrated great potential

**Table 1.** The list of test plants used in the host specificity tests for *Syphraea S. uberabensis* and the results, where X indicates positive results.

| Family | Species | Adults | Larvae | Eggs |
|---|---|---|---|---|
| Anacardiaceae | *Rhus sandwichensis* Gray | | | |
| | *Schinus terebinthifolius* Raddi | | | |
| | *Lithraea brasiliensis* Marchand | | | |
| Euphorbiaceae | *Manihot esculenta* Crantz | | | |
| | *Phyllanthus tenellus* Roxb. | | | |
| Lythraceae | *Lafoensia pacari* St. Hil. | | | |
| Melastomataceae | *T. herbacea* ( DC. ) Cogn. | X | X | X |
| | *T. cerastifolia* Cogn. | X | X | X |
| Monimiaceae | *Peumus boldus* Molina | | | |
| Myrtaceae | *Psidium cattleianum* Sabine (red form) | | | |
| | *Psidium cattleianum* Sabine (yellow form) | | | |
| | *Psidium guava* L. | | | |
| | *Campomanesia xanthocarpa* O. Berg. | | | |
| | *Eugenia uniflora* L. | | | |
| | *Eucalyptus grandis* W. Hill ex. Maiden | | | |
| Onagraceae | *Ludwigia* sp | | | |
| Poaceae | *Bambusa vulgaris*Schrad | | | |
| Rosaceae | *Pyrus malus* L. | | | |
| | *Pyrus communis* L. | | | |
| Thymelaeaceae | *Daphnopsis racemosa* Griseb | | | |

to be used in the control of *T. herbacea* due the extensive damage caused to the plant. The leaves were skeletonized removing completely the plant matter, leaving only the stem and vein structure. As a consequence, plant growth was reduced, and flowering and consequently seed production were prevented.

The consumed leaf area was higher by the larvae (third instar) that in the adult stage. The consumption difference was not significant because, on average, the larvae consumed less than half of a square centimetre more than the adults. The weekly consumption area of the adults was on average 1.15 $cm^2$, approximately 12.8% of the total leaf area, and the consumption of the larvae was on average of 1.28 $cm^2$, approximately 14.2% of the total leaf area.

In the plastic bottles experiment, it was observed that consumption of about 25% of the leaf was enough to dry it out and cause leaf drop. The plant showed high vulnerability to the *S. uberabensis* attack, which caused the defoliation of all of the plants and they all died, on average, after 4 weeks.

In the field, as in the laboratory, the leaves of both *Tibouchina* species demonstrated low or no regenerating capacity after the attack of the *S. uberabensis*, drying soon after a period of 2 weeks of the insect damage.

# Discussion

Laboratory and field investigations confirmed that *S. uberabensis* is strictly specific to the genus *Tibouchina* and therefore a safe biological control agent for *T. herbacea* in Hawaii. According to Harris (1971), the loss of mature leaves is normally most damaging to the plant, as these leaves represent the direct photosynthetic capacity of the plant. The attack by *S. uberabensis* is therefore meaningful, as no preferences based on the age of the leaves were found. As result of these studies and the potential of this insect as a biological control agent, 2000 insects were sent in 2005 to the Quarantine Service of USDA in Hawaii where further host-specificity tests are being conducted.

# Acknowledgements

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# Host-specificity testing of *Prospodium transformans* (Uredinales: Uropyxidaceae), a biological control agent for use against *Tecoma stans* var. *stans* (Bignoniaceae)

**A.R. Wood**[1]

*Summary*

Yellow bells, *Tecoma stans* (L.) Juss. ex Humb., Bonpl. & Kunth (Bignoniaceae), originally from Meso- and South America, is an emerging weed in the warm, moist regions of South Africa. The microcyclic, gall-inducing, rust fungus *Prospodium transformans* (Ellis & Ever.) Cummins (Uredinales: Uropyxidaceae) is a Neotropical pathogen of *T. stans*. It has been observed to be damaging to its host under natural conditions and is being considered as a biological control agent for use against this weed in South Africa. Two isolates were collected, one from Guatemala and the other from southern Mexico, and established and maintained in quarantine in South Africa. Host-specificity testing was conducted using both isolates against 14 species of Bignoniaceae (eight indigenous to southern Africa) and a further eight indigenous species in closely related families. No symptoms were produced on any of these species, except for small chlorotic spots on *Fernandoa magnifica* Seem. Sporulating galls were produced on all control *T. stans* plant leaves, petioles and stems. Leaves of the Bignoniaceae plants tested were examined microscopically at 7 days after inoculation. Fungal colonies of approximately 300 µm in diameter developed in control *T. stans* leaves, but the rust did not colonize any other species. Therefore, *P. transformans* is considered safe for introduction into South Africa, and permission for its release will be sought.

***Keywords***: South Africa, emerging environmental weed, yellow bells.

## Introduction

*Tecoma* Juss. (Lamiales, Bignoniaceae) is a genus of 14 species, mainly occurring in the Neotropics but with two species in Africa (Gentry, 1992). The genus can be divided into two groups, one with narrow tubular bird-pollinated flowers and the other with capanulate bee-pollinated flowers. The two African species are included in the former group, which is the more diverse. The latter group includes *Tecoma stans* (L.) Juss. ex Humb., Bonpl. & Kunth and three other more narrowly distributed segregate species (Gentry, 1992).

*T. stans* var. *stans* is a small tree with a widespread natural distribution, occurring throughout Mesoamerica and the Caribbean, as well as much of South America (Gentry, 1992). Within this range, it is morphologically variable, intergrading in places with the other two described varieties (var. *velutina* DC. and var. *angustata* Rehder; Gentry, 1992).

This plant is naturalized in South Africa, where it invades natural and disturbed vegetation and is therefore a declared weed (Henderson, 2001). Although not yet regarded as a major weed, it is considered to have the potential to invade a large proportion of the country (Nel et. al., 2004). It is currently increasing in abundance and has been chosen as a target of a biological control programme aimed at preventing it from emerging as a weed of national importance (Olckers, 2004).

*Prospodium* Arth. (Uredinales: Uropyxidaceae) is a Neotropical genus of about 50 species predominantly parasitizing members of the Bignoniaceae, with the rest on the Verbenaceae (Cummins and Hiratsuka, 2003). One species, *Prospodium tuberculatum* (Speg.) Arth., has been introduced into Australia for the biological control of *Lantana camara* L. (Tomley and Riding, 2002), and another, *P. tumefaciens* Lind., has been proposed as a potential agent for use against *Aloysia*

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*gratissima* (Gill. et Hook.) Troncose in the USA (Cordo and DeLoach, 1995).

Three *Prospodium* spp. have been recorded as occurring on *T. stans*, namely the macrocyclic *P. appendiculatum* (Wint.) Arth. and the microcyclic *Prospodium transformans* (Ellis & Ever.) Cummins and *P. elegans* (Schroet.) Cummins. The latter two are presumed to have been derived by a contraction of the life cycle of *P. appendiculatum* (Cummins, 1940). All three may be considered as potential biological control agents for use against *T. stans* in South Africa. One of these, *P. appendiculatum*, is adventitious in Brazil and is currently being assessed for its effectiveness as a biological control agent against *T. stans* in that country (Vitorino et. al., 2004).

In its native range (Caribbean basin, Guatemala, Mexico), *P. transformans* has only been recorded from *T. stans* var. *stans* and var. *velutina* (as *T. mollis* Humb., Bonpl. & Kunth) (Cummins, 1940). This rust fungus causes galls up to 3 cm in diameter on petioles, stems and seed pods, on which initially pycnia then telia develop. These are the only two stages of this species' life cycle. The teliospores may germinate as soon as they develop (Shuttleworth, 1953). Because of its known, limited host range and the damage that it causes to its host plant in its native range, studies were undertaken to assess the suitability of introducing *P. transformans* in South Africa for the biological control of *T. stans* var. *stans*. Results reported in this paper deal with host-specificity testing of *P. transformans* before seeking approval for release of this species in South Africa.

# Methods and materials

## Source and maintenance of cultures

Two isolates of *P. transformans*, originally collected from southern Mexico and Guatemala, were maintained in the quarantine glasshouses at ARC-Plant Protection Research Institute, Stellenbosch, South Africa, by repeated inoculation of potted *T. stans* plants. The plants were grown from seed collected in South Africa. These two isolates were kept in separate glasshouses to prevent cross-contamination. Plants were inoculated by dusting dry teliospores on the petioles and adaxial surfaces of immature leaflets (pinnae) using a small paintbrush, spraying the leaflets with water using an air brush or atomizer until very small droplets were visible to the naked eye, and then sealing the plants within a plastic bag. The inoculated plants were placed in an incubator at 18°C (Shuttleworth, 1953) for 48 h and then transferred to the quarantine glasshouses with a day/night temperature cycle of 25/19°C.

## Host-specificity testing

Plants of indigenous and locally cultivated species in the Bignoniaceae and other selected families of the Lamiales (Table 1) were inoculated in the same manner as above. Six plants of each species in the Bignoniaceae were inoculated, three using the southern Mexican and three using the Guatemalan isolates. Three plants of each species in the other families were inoculated with only the isolate from southern Mexico. The plants were then observed for gall development and sporulation for 1 month after inoculation. For every batch of plants inoculated, a plant of local *T. stans* was inoculated in the same manner, at the same time. Only if sporulating galls developed on these control plants were the results recorded, otherwise the plants were re-inoculated. Every plant was inoculated twice, the second time on new growth not previously inoculated.

## Microscopic examination

Two plants of each of the tested Bignoniaceae species were inoculated as above. Seven days after inoculation, two leaves from each plant were harvested and prepared for microscopic examination using the whole-leaf clearing and staining technique of Bruzzese and Hassan (1983). The stained leaves were examined at 400× magnification for penetration and development of mycelium by *P. transformans*. A control plant of local *T. stans* was included for each inoculation.

**Table 1.** List of plant species included in host-specificity testing of *Prospodium transformans*.

| Plant species | Origin |
|---|---|
| **Bignoniaceae** | |
| *Fernandoa magnifica* Seem. | Af |
| *Fernandoa sp.* | Af |
| *Jacaranda mimosifolia* D. Don. | e |
| *Kigelia africana* (Lam.) Benth | SA |
| *Macfadyena unguis-cati* (L.) A.H. Gentry | e |
| *Markhamia obtusifolia* (Baker) Sprague | Af |
| *M. zanzibarica* (Bojer ex DC.) K. Schum. | SA |
| *Podranea ricasoliana* (Tanfani) Sprague | SA |
| *Pyrostegia venusta* (Ker Gawl.) Miers | e |
| *Rhigozum obovatum* Burch. | SA |
| *Spathodea campanulata* P. Beauv. | Af |
| *Tecoma capensis* (Thunb.) Lindl. | SA |
| *T. stans* (L.) Juss. ex Humb., Bonpl. & Kunth var. *stans* | e |
| **Acanthaceae** | |
| *Duvernoia adhatodoides* E. mey. ex Nees | SA |
| *Mackaya bella* Harv. | SA |
| **Oleaceae** | |
| *Jasminum multipartitum* Hochst. | SA |
| **Schrophulariaceae** | |
| *Freylinia tropica* S. Moore | SA |
| *Halleria lucida* L. | SA |
| **Verbenaceae** | |
| *Lantana rugosa* Thunb. | SA |
| *Lippia rehmania* H. Pearson | SA |
| *Lippia scaberima* Sond. | SA |

*SA* Indigenous to South Africa, *Af* indigenous elsewhere in Africa, *e* exotic to Africa.

# Results

## Host-specificity testing

On the control *T. stans* plants, chlorotic flecks were visible on the leaf blades approximately 5 days after inoculation. Galls began to develop on these flecks soon after; small galls on the leaf blades (reaching approximately 5 mm in diameter after a month) but larger galls on the petioles or stems (up to 30 mm long). Pycnia developed on the galls beginning approximately 12 days after inoculation, and then telia appeared approximately 18 days after inoculation. No symptoms developed on any of the plant species tested, except for small chlorotic spots on *Fernandoa magnifica* Seem.

## Microscopic examination

Mycelium of *P. transformans* colonized an area of approximately 300 µm diameter for each infection in leaves of *T. stans* 7 days after inoculation, associated with the chlorotic flecks visible to the naked eye. No such mycelium was observed on any of the other Bignoniaceae species examined.

Small groups of dead epidermal cells were observed on leaflets of one *F. magnifica* leaf, and empty basidiospores were still attached to many of these. Small crystals were concentrated in the surrounding epidermal cells, and the underlying parenchyma cells appeared more densely distributed compared to surrounding areas. On other leaves of *F. magnifica*, no dead cells were observed, but areas of dense parenchyma cells and large crystals occurred. No mycelium was observed in or around these areas. The differences between these leaf reactions were probably due to differences in leaf age.

Single dead cells at the point of penetration were observed in *Tecoma capensis* (Thunb.) Lindl., indicating a hypersensitive reaction. Neither mycelium nor any plant reaction was visible for *Fernandoa* sp., *Jacaranda mimosifolia* D. Don., *Macfadyena unguis-catii* (L.) A.H. Genrty, *Markhamia obtusifolia* (Baker) Sprague, *Markhamia zanzibarica* (Bojer ex DC.) K. Schum., *Podranea ricasoliana* (Tanfani) Spraque, *Pyrostegia venusta* (Ker Gawl.) Miers, *Rhigozum obovatum* Burch. or *Spathodea campanulata* P. Beauv.

# Discussion

The Bignoniaceae is a small family in southern Africa, having only 16 species in nine genera in this region (Diniz, 1988; Smithies, 2003). Representatives of six of these genera were tested and found not to become infected with *P. transformans*, including *T. capensis*, a congener of the target weed. It was observed microscopically that a hypersensitive reaction occurred in *T. capensis*. Additionally tested members of the Bignoniaceae native to South America (cultivated and/or naturalized in South Africa) and indigenous representatives of other families in the Lamiales all showed no symptoms of infection. The only plant tested that showed any symptoms (chlorotic spots) was *F. magnifica*. Microscopic examination revealed no fungal mycelium associated with these chlorotic spots; rather, dense parenchyma and accumulated crystals occurred. These probably indicate that the chlorotic flecks were due to a plant defence reaction.

Because of the high level of host specificity demonstrated, it is considered that this rust fungus is safe for introduction into South Africa for the biological control of *T. stans* var. *stans*. This conclusion is supported by the narrow host range recorded in its native distribution (Cummins, 1940). Permission for its release will be sought from the relevant authorities.

# Acknowledgements

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# Study on the herbicidal activity of vulculic acid from *Nimbya alternantherae*

**M.M. Xiang, L.L. Fan, Y.S. Zeng and Y.P. Zhou[1]**

*Summary*

The detached leaves of 28 species of plants and the plants of alligator weed were tested for toxic activity of vulculic acid isolated from *Nimbya alternantherae*. Results indicated that the toxin was non-host-specific and could cause leaf blight and withering of alligator weed. The effect of the toxin on the ultrastructure of leaf tissue of alligator weed was studied by treating the mature leaves with the toxin. It was shown that the damage on leaf tissue included plasmolysis and vacuolation in cells, lamellae disorder and vacuolation in chloroplasts as well as disappearance of ridges and vacuolation in mitochondria after treatment with vulculic acid at a concentration of 50 μg/ml for 12 h. These results suggest that the target sites for vulculic acid action may be the plasma membranes, the lamellae of chloroplast and the ridges of mitochondria of the alligator weed leaf.

***Keywords***: mycotoxin, alligator weed, ultrastructure, pathogenic mechanism.

## Introduction

Alligator weed, *Alternanthera philoxeroides* (Martius) Grisebach, is widely known as a serious exotic weed. Some progress has been achieved in the biological control of this weed with plant pathogenic fungi and their metabolites. Pathogenic fungi reported on alligator weed include *Rhizoctonia solani* Kühn (Singh and Devi, 1991), *Colletotrichum* sp. (Tan and Gu, 1992), *Cercospora alternantherae* Ellis & Langlois (Barreto and Torres, 1999) and *Nimbya alternantherae* (Holcomb & Antonopoulus) Simmons & Alcom (Xiang *et al.*, 1998; Barreto and Torres, 1999). The latter two species are considered to have a potential as biological control agents for alligator weed (Barreto and Torres, 1999; Barreto *et al.*, 2000; Xiang *et al.*, 2002a). Moreover, Wan *et al.* (2001) found that the crude preparation of the toxin from *Alternaria alternata* (Fr.) Keissler was strongly toxic to the leaves of the alligator weed. The metabolic product of *Fusarium* sp. could also induce leaf lesions and withering of the weed (Tan *et al.*, 2002).

*N. alternantherae* can cause purplish leaf spots and defoliation (Barreto and Torres, 1999; Xiang *et al.*, 2002a) and is a highly host-specific pathogenic fungus (Xiang *et al.*, 2002a). Xiang *et al.* (2002b) found that the filtrate of this fungus had herbicidal activity and could inhibit radicle growth in sorghum, and Zhou *et al.* (2006) isolated vulculic acid from the filtrate. To provide a foundation for developing a biochemical herbicide, the herbicidal activity of this toxin was studied and is reported in this paper.

## Materials and methods

### Materials

**Toxin material:** Vulculic acid was isolated from the filtrate of *N. alternantherae* using the method of Zhou *et al.* (2006), which was slightly modified. *N. alternantherae* was cultured in modified Fries (Xiang, 2005) at 28°C, 200 rpm for 7 days, and the culture liquid was filtered. Methanol was added into the filtrate in the volume ratio of 1:3, stirred, then filtered and evaporated at 60°C to get a mush. The crude product was extracted with ethyl acetate from the mush, and dissolved with benzene and acetone (in volume ratio of 1:1) at 60°C and filtered. After crystallizing and re-crystallizing in an ice-bath two to three times with benzene and acetone in 1:1 ratio, crystals with a few impurities were mixed with silica gel in a mass ratio of 1:1 and put into a column of 300 × 15mm, eluting with benzene and acetone (1:1). The eluting liquid was evaporated at 60°C until 30 to 40 ml remained and then re-crystallized in an ice-bath. The toxin obtained was dried in a vacuum desiccator, and its purification was evaluated by high-performance liquid chromatography and infrared spectra (IR).

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**Plant material:** Alligator weed was grown in nutritional liquid that is commonly used for leafy vegetable production. The other 27 species of plants tested for toxic activity were all seedlings grown in a greenhouse or taken from the field (see Table 1).

**Table 1.** Sensitivity of the plants tested to vulculic acid.

| Plant species | Concentration of the toxin (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 50 | 30 | 10 | 5 | CK |
| Red pepper (*Capsicum annuum* L.) | + | – | – | – | – |
| Tomato (*Lycopersicon esculentum* Mill) | + | + | + | – | – |
| Eggplant (*Solanum melongena* L.) | – | – | – | – | – |
| Spinach (*Spinacia oleracea* L.) | + | – | – | – | – |
| Water cress (*Nasturtium officinale* R.Br.) | – | – | – | – | – |
| Radish (*Raphanus sativus* L.) | – | – | – | – | – |
| Gynura (*Gynura bicolor* L.) | + | + | + | – | – |
| Yerbadetajo (*Eclipta prostrate* L.) | + | + | + | + | – |
| Red tasselfpower [*Emilia sonchifolia* (L.) DC.] | – | – | – | – | – |
| Crowndaisy oxeyedaisy (*Chrysanthemum coronarium* L.) | – | – | – | – | – |
| Lettuce (*Lactuca sativa* var. *crispa* L.) | – | – | – | – | – |
| China crabdaisy [*Wedelia trilobata* (Osb.) Merr.] | + | + | – | – | – |
| Japanese youngia [*Youngia japonica* (L.) DC.] | – | – | – | – | – |
| Celery (*Apium graveolens* L.) | + | + | + | – | – |
| Coriander (*Coriandrum sativum* L.) | + | + | – | – | – |
| Carrot (*Daucus carota* L. var. *sativus* DC.) | + | – | – | – | – |
| Pea (*Pisum sativum* L.) | + | – | – | – | – |
| Sour dallisgrass (*Paspalum conjugatum* Berg.) | + | – | – | – | – |
| Corn (Zea mays L.) | – | – | – | – | – |
| Sweet potato (*Ipomoea batatas* Lam.) | + | + | + | – | – |
| Dashen [*Colocasia esculenta* (L.) Schott] | + | + | – | – | – |
| Cucumber (*Cucumis sativus* L.) | + | + | – | – | – |
| Nutgrass cypressgrass (*Cyperus rutundus* L.) | + | + | + | – | – |
| Red woodsorrel (*Oxalis corymbosa* DC.) | – | – | – | – | – |
| Asia plantain (*Plantago asiatica* L.) | + | + | – | – | – |
| Alligator weed [*A. philoxeroides* (Martius) Grisebach] | + | + | + | + | – |
| Thorny amaranth (*Amaranthus spinosus* L.) | – | – | – | – | – |

Note: "+" means sensitive, "-" means insensitive

## Methods

**Effective range of the toxin:** The purified toxin was diluted to give concentrations of 50, 30, 10 and 5 μg/ml with double-distilled water. The healthy leaves of the plants were washed with tap water and then three times with double-distilled water, dried on sterile filter paper, cut into 0.5 ×0.5 cm pieces and then were placed into test tubes with 2 ml of the toxin solution. Ten plant pieces were added to each tube, and then was a duplicate for each concentration. The tubes with solution and pieces were put into an illuminated incubator at 25°C for 24 h, and pathological changes were recorded.
**Effect of the toxin on alligator weed plant:** The purified toxin was diluted to concentrations of 300, 200, 100 and 50 μg/ml with distilled water. Alligator weed stems that had just begun to develop roots were dug up in the field, washed in tap water and grown in nutrient solution for 3 to 4 days. Then, the plants were washed three times with distilled water, dried on sterile filter paper and transplanted into tubes containing 10 ml of the different toxin dilutions. Three plants were included in each tube, and tubes were duplicated for each concentration. Distilled water was used in control tubes. The treated plants were grown at room temperature for 24 h, and then pathological changes were recorded.
**Effect of the toxin on the ultrastructure of alligator weed leaf:** The purified toxin was diluted to a concentration of 50 μg/ml with double-distilled water. Healthy mature leaves of alligator weed were washed with tap water and then three times with double-distilled water, dried on sterile filter paper and cut into pieces of 2 to 3 mm transversely. The pieces were placed into test tubes with 2 ml of the toxin solution and decompressed for 20 min. Then, the tubes with solution and pieces were put into an illuminated incubator at 25°C for 12 h. Double-distilled water was used in the control tube.

The samples were fixed with 2.5% glutaraldehyde, then with 1% osmic acid. After being dehydrated using a standard method, the samples were embedded in Epon 812. Microtome sections were dyed with uranium acetate, then lead citrate and observed through FEI-Tecnai 12 transmission electron microscope.

# Results

## Effective range of the toxin

Vulculic acid was a non-host-specific toxin. It had toxic activity towards many plants from different families or genera, including alligator weed, after treatment for 24 h under almost all of the concentrations tested (Table 1). It caused brown blight on the leaf pieces.

## Effect of the toxin on alligator weed plant

The toxin inhibited root growth and induced wilt of alligator weed plants after treatment for 24 h under all of the concentrations tested (Fig. 1A, B).

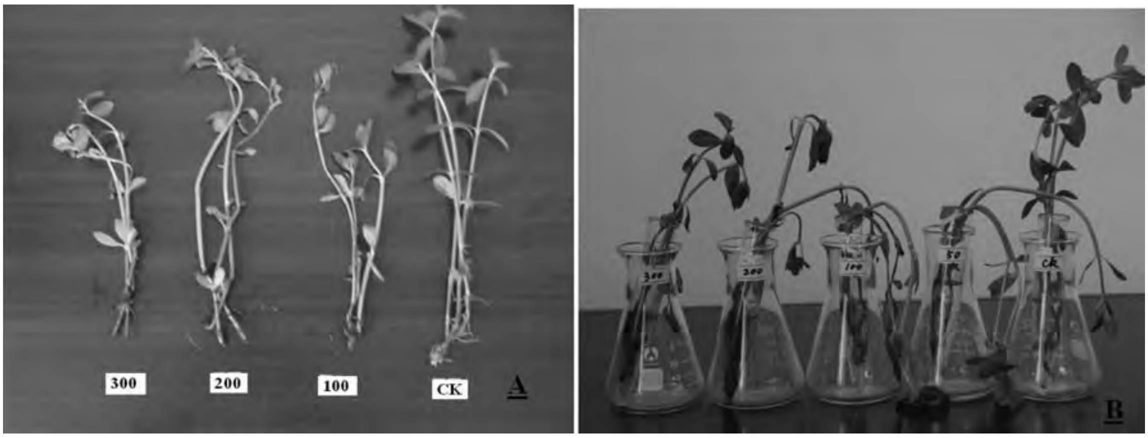


**Figure 1.** **A** Roots of alligator weed treated with the toxin for 24 h. **B** Plants of alligator weed treated with the toxin for 24 h.

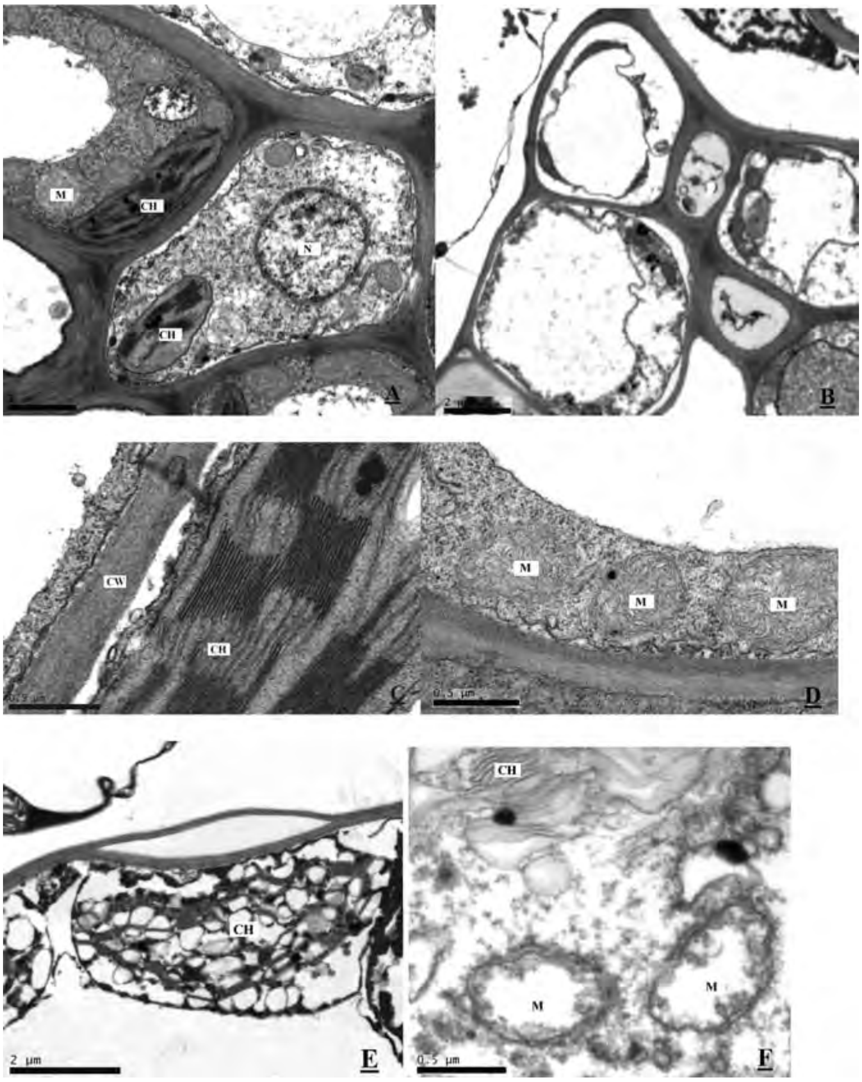


**Figure 2.** **A** Normal ultrastructure in the alligator weed control leaf. **B** Plasmolysis and vacuolated cells in the treated leaf with the toxin. **C** Normal lamellae of chloroplast in the control leaf. **D** Normal structures of mitochondria in the control leaf. **E** Disordered lamellae and vacuolated chloroplasts in the treated leaf with the toxin. **F** Vacuolated mitochondria and disordered lamellae of chloroplast in the treated leaf with the toxin. *CH* Chloroplast, *M* mitochondria, *N* nuclei, *CW* cell wall.

### Effect of the toxin on ultrastructure of alligator weed leaf

In tissue samples after treatment with vulculic acid, at a concentration of 50μg/ml for 12h, the plasmalemma of leaf cells was invaginated and detached from the cell wall in almost all places, and the cells became vacuolated. However, the plasmalemma did not appear ruptured (see Fig. 2B). Chloroplasts were severely damaged with disorder lamellae and a lot of vacuoles in the treated tissue with the toxin (Fig. 2E). Mitochondria from leaf cells treated with the toxin showed significant disorganization, such as the decrease of ridges and the vacuolation of mitochondria (Fig. 2F).

## Discussion

The toxin, vulculic acid, isolated from *N. alternantherae*, was reported to inhibit the pollen germination of black pine, *Pinus thunbergii* Parl., by up to 85.3% at a concentration of 10 mg/l (Kimura *et al.*, 1991). Before our study, its toxicity to other plants had not been reported. The preliminary screening results showed that vulculic acid is a non-host-specific toxin and could inhibit root growth and induce wilt of alligator weed. Thus, the advantage of vulculic acid as an herbicide compared to *N. alternantherae* lies in its wider host range and better prospect for product development.

In this study, vulculic acid was toxic to the plasmalemma, the lamellae of chloroplast and the ridges of mitochondria of alligator weed leaf cells after treatment at a concentration of 50 μg/ml for 12 h. These results suggest that the target sites for the toxin action may be on the plasma membranes, the lamellae of chloroplast and the ridges of mitochondria of alligator weed leaf. However, this is the first and preliminary study on the ultrastructural effect of vulculic acid on plant tissues. Further studies are required to determine which one of the three target sites is damaged first and the minimum concentration of toxin needed to cause the damage.

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# Optimization of water activity and placement of 'Pesta-*Pseudomonas fluorescens* BRG100'—biocontrol of green foxtail

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*Pseudomonas fluorescens* BRG100 was selected from earlier screening studies for pre-emergent bioherbicidal activity to green foxtail and wild oat. A granular formulation, Pesta, has been developed to deliver *P. fluorescens* BRG100. Delivery and placement of sufficient numbers of BRG100 to inhibit or suppress germination of the weed is one of the key challenges in bioherbicide product development. However, optimization of BRG100 survival, placement and dispersion from the Pesta granule in the target zone has not been fully established. Increased shelf-life of BRG100 in Pesta may be acquired by increasing BRG100 cell membrane integrity, optimizing the water activity of the granules ($a_w$), a useful measure of the free (unbound) water that is available for use by microorganisms. Addition of maltose, 3% *w*/*w*, reduced survival of BRG100 in peat culture and in Pesta granules prepared from peat powder cultures as compared to peat powder culture and resulting Pesta without maltose. Survival of BRG 100 in Pesta was greatest with the water activity ($a_w$) adjusted to 0.2 as compared to 0.5 and 0.8 $a_w$. Placement of Pesta in-row and side-banded with green foxtail was examined in a greenhouse study. Evidence of phytotoxin damage to green foxtail by Pseudophomins A and B was observed.

# Impact of natural enemies on the potential damage of *Hydrellia* sp. (Diptera: Ephydridae) on *Egeria densa*

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*Egeria densa* Planchon (Brazilian Elodea or Brazilian waterweed) is a South American submerged perennial in the Hydrocharitaceae that has become a weed in North America, Australia, New Zealand, South Africa and parts of Asia and Europe. It crowds out other plant species by forming dense stands, negatively affecting the native biota, as well as water sports, fishing, navigation, delivery of irrigation water and hydropower production. The larva of *Hydrellia* sp. (Diptera: Ephydridae) from Argentina feeds on the mesophyl, producing chlorosis (bleaching) of two to three whorls per larva, and mining the stem in between them. Under laboratory conditions, a single gravid female can cause the defoliation of whole stems. In the field, this insect has several natural enemies that attack the larvae and the pupae. We discuss its potential impact on the weed under an 'enemy release' situation, considering *Hydrellia* has both specific and generalist natural enemies.

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# Towards to study of the sunflower broomrape fungi disease in Georgia

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The parasite plant, sunflower broomrape, *Orobance cumana*, is a major pest and is widely spread in sunflower production regions of East Georgia. Experiments were conducted on influence of the introduced fungus, *Fusarium oxysporum* f. sp. *orthoceras* (FOO) on *O. cumana* and its host sunflower strain 'Donskoy-60'. These investigations indicate that FOO is a potent biological agent to control against *O. cumana* in conditions experienced in Georgia, and it is an important component in integrated pest management for sunflower management.

# Biological control of *Imperata cylindrica* in West Africa using fungal pathogens

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*Imperata cylindrica* (cogon grass), a noxious, rhizomatous grass with a pan-tropical distribution represents one of the most serious constraints to crop production and poverty alleviation in West Africa. The fungi, *Bipolaris sacchari* and *Drechslera gigantea*, have shown potential as bioherbicides to control cogon grass (var. major) in the southeastern USA. Biological control may however prove to be ineffective if the West African cogon grass (var. *africana*) is genetically heterogeneous from the southeastern USA cogon grass. The objectives of this study are to assess the genetic diversity between the West African and southeastern USA cogon grass populations and to determine the virulence of the southeastern USA and West African isolates of *B. sacchari* and *D. gigantea* on the West African cogon grass population. A further objective is to determine the potential of three biotrophs: two rust fungi, *Puccinia imperatae* and *Puccinia fragosoana*, and a head smut, *Sporisorium schweinfurthiana*, associated with cogon grass in South Africa, where cogon grass is not a weed, for control of cogon grass. Interim results indicate that there are no differences between the USA and West African fungal isolates in terms of their virulence on the var. africana. The genetic variation results and the implications for fungal biocontrol on cogon grass in West Africa will be discussed.

# Impact of *Ischnodemus variegatus* (Hemiptera: Blissidae) on the invasive grass *Hymenachne amplexicaulis* in Florida

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Invasions of exotic grasses constitute a major threat to aquatic ecosystems. West Indian Marsh Grass, *Hymenachne amplexicaulis* (Rudge) Nees, which is native to South America, is considered a major environmental weed in southeastern USA and Australia. In Florida, an adventive insect was recently found causing severe damage to *H. amplexicaulis*. This insect was identified as *Ischnodemus variegatus* (Hemiptera: Blissidae) and is considered native to South America. The host range of this herbivore and its potential to control *H. amplexicaulis* were evaluated under laboratory, greenhouse and field conditions. We tested 60 plants under no-choice conditions for development and five plants for oviposition of the insect. *I. variegatus* had higher survival from nymph to adult on *H. amplexicaulis* than on other tested plants. Development to the adult stage also occurred on *Panicum hemitomon*, *Panicum anceps*, *Paspalum urvellei* (all Poaceae) and *Thalia geniculata* (Marantaceae). Oviposition choice tests demonstrated that *I. variegatus* females will lay eggs on several non-target grasses. Greenhouse experiments demonstrated that feeding damage of *I. variegatus* reduces the growth rate, chlorophyll levels and biomass of *H. amplexicaulis* seedlings. Field sampling of naturally occurring populations in Florida indicated that *I. variegatus* density, under favourable climatic conditions, increase during the summer and can experience outbreaks that severely reduce *H. amplexicaulis* survival and reproduction.

# Ecological basis for biological control of *Arundo donax* in California

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*Arundo donax* invades California riparian areas and is a target for biological control. Candidate agents have been identified, but their eventual release will depend upon evidence that damage is substantial and novel. As part of a program comparing Arundo growth traits and damage in California and the Mediterranean region (its presumed origin), we documented the presence in southern California of *Tetramesa romana* (Walker) (Hymenoptera: Eurytomidae), the same stem-boring sawfly being tested in quarantine laboratories for future introduction. Primary or secondary shoots <10 cm diameter are occupied, with densities up to 34 larvae per 100 cm of culm, and mortality of secondary shoots is common. The wasp has been shown to infect new hosts under experimental field conditions, so we have an opportunity to test its efficacy and host range without the artefacts that plague standard quarantine testing. Field studies continue on both continents to document life cycles and impacts and determine whether this insect can utilize alternative hosts such as *Phragmites australis* and other native or economic grasses. If safety can be shown, this wasp may be amenable to re-distribution to other infested ecosystems in the western USA.

# Biology and host specificity of *Puccinia arechavaletae*, a potential agent for the biocontrol of *Cardiospermum grandiflorum*

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*Cardiospermum grandiflorum* (balloon-vine), a subtropical climber from South America, is an emerging weed in some regions in Southern Africa. It is a highly invasive, vigorous climber that invades mostly forest margins and watercourses in sub-tropical areas such as KwaZulu-Natal and the Kruger National Park in Mpumalanga, South Africa. Robust stems with tendrils enable *C. grandiflorum* to climb and form a dense canopy, which completely smothers the underlying indigenous vegetation. The fruit capsules are carried by wind and float freely on water, dispersing the plant along waterways, and the hard seeds result in extended seed longevity in the soil. It is classified as a category 1 weed in South Africa, meaning that it is prohibited and must be controlled or eradicated where possible, and it was consequently targeted for biological control. An isolate of the rust fungus, *Puccinia arechavaletae*, was collected in South America, from a biotype of *C. grandiflorum* that matches the morphology of the South African weed. This isolate was successfully established on *C. grandiflorum* in quarantine laboratories at the ARC-PPRI in South Africa. Host-range testing showed that this isolate of *P. arechavaletae* is specific to *C. grandiflorum*, making it a promising agent for the biocontrol of this environmental weed.

# Potential for host-specific biological control agents at population/subspecies level?

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Host-specificity testing and post-release evaluations of biological control agents show that closely related plant species (same genus) are most likely to be at risk of non-target attack. Most biocontrol programs aim for species-specific control agents, yet in the program targeting invasive *Phragmites australis* in North America, the existence of a native subspecies (*Phragmites australis americanus*) requires specificity at the subspecies level. Is it realistic to expect to find herbivores that distinguish between native and introduced *P. australis*? Field surveys in North America found native specialist herbivores attacking only native *P. australis*, suggesting distinct differences between native and introduced genotypes that are recognizable by herbivores. Preliminary multiple-choice oviposition tests and no-choice larval development tests with several herbivore species considered as potential biological control agents in Europe showed the ability of these herbivores to complete development on native and introduced *P. australis* genotypes. However, we also found a preference of these herbivores for introduced *P. australis*. These preferences together with differences in plant morphology may allow a biocontrol program to proceed even if no subspecies-specific control agents are available.

# Combined effects of herbicides and rust fungi on *Rumex obtusifolius*

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The rust fungus, *Uromyces rumicis*, has been investigated as a potential biocontrol agent for the perennial grassland weed *Rumex obtusifolius* in Europe. Although the rust infection reduces plant growth, it does not kill the plant, cannot infect the young leaves and causes only a moderate reduction in seed production. Thus, it is unlikely to be a successful biocontrol agent on its own. In this study, we investigated the possibility that low doses of herbicides might act as synergists to the rust. Out of several herbicides tested, asulam, thifensulfuron-methyl and dicamba increased *Uromyces* spore germination when applied at 1/300th recommended concentration, and the first two also had a positive effect at 1/150th concentration. When applied on *R. obtusifolius* at these concentrations, both the rust and herbicides had significant effects alone. Together they often had an additive, but not a synergistic effect.

# Host-specificity and potential of *Kokujewia ectrapela* Konow for the control of *Rumex* spp.

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The host specificity and food consumption of *Kokujewia ectrapela* Konow (Hym., Argidae) were studied to evaluate the potential of this sawfly as a non-endogenous biological control agent of *Rumex* spp. in Australia. This oligophagous, multivoltine sawfly is an indigenous species on *Rumex* spp. (Polygonaceae) in Russia, Transcaucasia and Iran. Results of no-choice feeding tests with second instars on 27 plant species belonging to 13 families showed that *K. ectrapela* completed its life cycle mainly on plants of *Rumex* and occasionally fed on *Polygonum persicaria* L. Under laboratory conditions, weights of consumed food by larvae were measured at 25°C. Feeding activity of three larval instars of *K. ectrapela* on *Rumex obtusifolius* L. were investigated. Weight of consumed leaves differed between instars. During the 3 days of first instar development, one larva consumed 0.041 ± 0.001 g of *R. obtusifolius* leaves. The next two instars, with durations of 4 and 5 days, consumed significantly larger amount of leaves, viz., 1.227 ± 0.006 and 3.058 ± 0.014 g, respectively. Total weight of consumed leaves by all three instars of a single larva, during 12 days of the developmental time, amounted to 4.310 ± 0.01 g.

# Growth and phenology of three *Lythraceae* species in relation to feeding by the leaf beetles, *Galerucella* spp.

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Previous studies have characterized the development of *Galerucella calmariensis* and *Galerucella pusilla* on *Lythrum salicaria* and on the non-target *Lythraceae* species, *Lythrum alatum* and *Decodon verticillatus*. The impact of Galerucella on these species, when grown in outdoor mesocosms that more closely mimics ecological host range, has not been reported. The first objective of this study was to compare the growth and seed capsule production of *L. salicaria*, *L. alatum* and *D. verticillatus*, with and without Galerucella. With *L. salicaria*, larval feeding on apical and lateral shoot buds resulted in fewer seed capsules compared to control plants. No measured plant growth or reproductive parameters were reduced as a result of Galerucella feeding on *D. verticillatus*. Presence of Galerucella on *L. alatum* resulted in a reduction of seed capsules in 1 year of a 2-year study. A second objective of our study was to compare the phenology of the three *Lythraceae* species in relation to that of Galerucella. In the northern USA, flowering and seed development in *D. verticillatus* occurred a month later than in *L. salicaria* or *L. alatum*. The delayed flowering of *D. verticillatus* resulted in avoidance of shoot meristem feeding damage caused by the first generation of Galerucella.

# *Corynespora cassiicola* f. sp. *benghalensis*, a new natural enemy of *Commelina benghalensis*: infection parameters

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*Commelina benghalensis* (wandering Jew) is a herbaceous plant from Asia, which became one of the worst crop invaders after its recent arrival in Brazil, particularly in soybean and coffee. A survey aimed at discovering fungal pathogens that might already be established in Brazil revealed a limited mycobiota. Among the fungi that were collected was the eye-spot fungus *Corynespora cassiicola*. This is usually regarded as a polyphagous pathogen, but a host-range evaluation has shown that the strain that attacks *C. benghalensis* is a highly specific *forma specialis* and is sufficiently damaging to allow further considerations on its use as a mycoherbicide. Growth, sporulation and spore germination were evaluated under different conditions. The fungus was insensitive to light regimes. Optimum sporulation was between 25–30°C, whereas optimal conidial germination was between 20–25°C. The effect on disease severity of a combination of different dew periods and temperatures was tested, and the level of damage was proportional to the duration of the dew period, independent of temperature. Delays to the onset of dew period after inoculum application were also tested, and levels of disease severity were still high with delays of up to 24 h.

# Potential use of *Trichilogaster acaciaelongifoliae* as a biocontrol agent of *Acacia longifolia* in Portugal

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*Acacia longifolia* (long-leafed wattle) was introduced into Portugal more than 150 years ago to bind coastal dunes. Its distribution has increased greatly after fire events, and it is now one of the worst invasive plant species along the Portuguese coast. *Trichilogaster acaciaelongifoliae* (an Australian gall wasp) is being tested as a potential biological control agent of *A. longifolia*. If released, this would be one of the first planned introductions of a classical biological control agent against an environmental weed species in Europe. *T. acaciaelongifoliae* is a monospecific bud-galling pteromalid wasp that prevents its host plant from flowering normally and deforms vegetative growth. Seed production by *A. longifolia* has been reduced by more than 90% since the introduction of the wasp into South Africa in 1987. In specificity tests, females are confined on a set of test-plant species. Flower and vegetative buds are then dissected to detect eggs. Species on which eggs are found will then be the subject to larval development tests. The results so far have been promising. At the same time, climate studies are being undertaken to determine whether any regions of Portugal are unsuitable for *T. acaciaelongifoliae* and how best to move the wasps from the southern to the northern hemisphere.

# *Diclidophlebia smithi* (Hemiptera, Psylloidea): a potential biocontrol agent for *Miconia calvescens*

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*Miconia calvescens* (Melastomataceae) is a shrub or small tree native from the Neotropics that became an aggressive invader of natural ecosystems of the Pacific Islands after its introduction as an ornamental. Intensive searches for insects and pathogens to be used as biocontrol agents were conducted in its native range. Among the insects collected in Brazil, the newly described species was *Diclidophlebia smithi* (Hemiptera: Psyllidae),, which is often found attacking terminal buds, inflorescences and fruits of *M. calvescens*. It appeared to be causing significant impact on its host in the field. Population density of *D. smithi* was recorded for 2 years at three different localities in the state of Minas Gerais (Brazil). It occurs throughout the year, but its population is more abundant between April and October (the dry and cool autumn–winter period). No parastioid was found attacking *D. smithi*, and the sole significant natural enemy of this psyllid was a predatory Syrphidae larva. *Diclidophlebia* sp. n. has five nymph instars during its life cycle, which takes between 40 and 67 days. Preliminary specificity tests have indicated that *D. smithi* is highly host-specific, attacking only *M. calvescens*. Impact studies have shown that *D. smithi* affects significantly shoot development and flower and fruit bearing in *M. calvescens.*

# Supplementary host-specificity testing of *Puccinia melampodii*, a biocontrol agent of *Parthenium hysterophorus*

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*Parthenium hysterophorus*, native to South and Central America, is an invasive weed in KwaZulu-Natal, Mpumalanga and the northwest provinces of South Africa. The micro-cyclic rust fungus, *Puccinia melampodii*, has been successfully used in Australia and is being considered for release in South Africa. As this rust was subjected to comprehensive host-specificity testing in Australia, only supplemental testing was necessary. Testing to five selected South African sunflower cultivars and three out of eight indigenous *Heliantheae* (Asteraceae) species was undertaken. Plants were inoculated with basidiospores of *P. melampodii* and incubated at 25°C for 24 h. For each sunflower cultivar, three plants were tested in four replications, two replications for the indigenous plants, and three *P. hysterophorus* plants were included as control in every replication. Rust symptom development was monitored, and no symptoms developed on the sunflower plants and the indigenous Heliantheae, except for a few pustules that developed on two *Spilanthes mauritiana* plants in one replication. All *Parthenium* control plants were heavily infected. The conclusion was therefore made that the tested plant species and the sunflower cultivars are unlikely to be infected by *P. melampodii* in the event that this agent is released to control *Parthenium* in South Africa.

# Is *Prosopis* meeting its match in Baringo?

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The genus *Prosopis* is native to arid and semi-arid regions of Asia, Africa and America. Neotropical species, such as *Prosopis juliflora* and *Prosopis pallida*, have been introduced worldwide for multi-purpose use and their ability to survive poor conditions. *Prosopis* introductions into Kenya occurred mainly in 1980s, and it has since spread to neighbouring areas threatening the livelihoods of humans and ecosystems. In response, Food and Agriculture Organization supports a project to manage *Prosopis*. One objective is to introduce, test and release the *Prosopis* seed-feeding bruchid *Algarobius prosopis* from South Africa. The bruchid is undergoing specificity tests in quarantine. This beetle was imported on the assumption that *Prosopis* is spreading because it is outside its natural range and lacks natural enemies to regulate its population. In an effort to understand the ecology of *Prosopis*, we assessed the biodiversity (arthropods and microsorganisms) associated with *Prosopis* at Baringo, Kenya. There an indigenous insect fauna is associated with *Prosopis*. Some of these insects cause significant damage to the trees reducing reproductive potential and timber value. Can these insects be incorporated in a management strategy for controlling *Prosopis*? Studies to determine the relationship between the insects, *Prosopis* and the indigenous flora and to clarify the status and genetic variation of the *Prosopis* species and their assumed hybrids are underway.

# A lace bug as biological control agent of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae): an unusual choice

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*Tingis grisea* is a univoltine sap-feeding lace bug distributed throughout Central and Southern Europe and the Middle East reportedly associated with the genus *Centaurea*. During 2002, high *T. grisea* infestation levels were recorded on one yellow starthistle population in Eastern Turkey. Field observations showed that significant damage was caused to the host plant especially when individuals were feeding on the same plant in large numbers. Life cycle and biology observations allowed assessing the duration of the five nymphal stages of *T. grisea* in laboratory conditions as well as female fecundity and longevity. Starvation and oviposition no-choice tests were carried out to determine the host-specificity level of the insect. Results showed a clear oligophagous behaviour closely restricted to the genus *Centaurea*. In addition, among the three species on which full larval development was ascertained (*Centaurea solstitialis*, *Centaurea sulphurea* and *Centaurea cyanus*), yellow starthistle was clearly preferred in terms of number of eggs laid and number of adults obtained.

# Potential biological control of *Lantana camara* in the Galapagos using the rust *Puccinia lantanae*

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Laboratory experiments were carried out in England to test the specificity and environmental requirements of a Peruvian isolate of the fungus, *Puccinia lantanae* Farlow, known to attack the invasive plant *Lantana camara* L., a serious problem in Galapagos. Eight species of plants representing five families were inoculated with the fungus and kept in a dew chamber for 48 h. *Lantana peduncularis* Andersson and *L. camara* were sourced from Galapagos; other species related to *Lantana* were sourced from other places. Dew periods of 5, 8, 11, 14 and 20 h were tested to determine the period necessary for basidiospore formation and host infection. Only *L. camara* from Galapagos and Peru developed visible symptoms 6 days after inoculation, and after 15 days, sori were fully developed. No non-target species developed macroscopic symptoms. Most importantly, the rust did not attack the closest host relative from Galapagos, the endemic *L. peduncularis*. Eight hours in the dew chamber was enough to induce basidiospore formation and host infection, but times up to 20 h induced progressively more sori. Although we have not completed yet the experiments to determine the host-range specificity, *P. lantanae* shows a promise as a biocontrol agent for *L. camara* in Galapagos.

# Biology and host specificity of *Puccinia conoclinii* for biocontrol of *Campuloclinium macrocephalum* in South Africa

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*Campuloclinium macrocephalum* (pompom weed) is a perennial herb, which was presumed to be brought into South Africa for ornamental purposes. Initially, it could only be found in disturbed sites such as roadsides, but eventually, it spread to wetlands, open savanna and grasslands and is very prominent in the Gauteng Highveld. During summer, numerous annual shoots are produced. During winter, it survives as a rootstock in a dormant state underground. Due to prolific seed production and its survival abilities, this plant is multiplying and spreading rapidly. The ideal control method would be to use a biocontrol agent to damage the aerial parts of the plant. This will weaken the plant, reduce seed production and will deplete the nutrients stored in the roots. A rust fungus, which was tentatively identified as *Puccinia conoclinii* (only urediniospores observed), was collected from *C. macrocephalum* in Northern Argentina and introduced into the quarantine laboratories at the ARC-PPRI, Stellenbosch. Host-specificity testing revealed *C. macrocephalum* to be the only host of this rust isolate, and these results were confirmed by microscopic examination on the host and other closely related plant species.

# Status of tree of heaven, *Ailanthus altissima*, in Virginia, USA and quarantine evaluation of *Eucryptorrhynchus brandti* (Harold) (Coleoptera: Curculionidae), a potential biological control agent

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Tree of heaven (TOH), *Ailanthus altissima* (Mill.) Swingle, is an imported invasive weed tree from China that has become established throughout much of continental USA. It colonizes disturbed forest sites and often out-competes native vegetation. Short-term cultural and chemical controls of this weed are expensive and have limited efficacy. Two curculionid species, *Eucryptorrhynchus brandti* (Harold) and *E. chinensis* (Olivier), are primary mortality agents of *A. altissima* in China and have no other known hosts. The objectives of our project are (1) to assess the pest status of *A. altissima* in Virginia and (2) to evaluate *E. brandti*, the more numerous of the two species, for its potential as a biological control agent. A statewide survey showed significant presence of TOH but no native herbivores with potential of controlling it. Economic analysis of mechanical and chemical control indicates biological control to be an attractive alternative. *E. brandti* requires live trees for development. Therefore, quarantine studies have focussed on developing a rearing technique and testing host specificity on native plants approved by the Technical Advisory Group for Biological Control Agents of Weeds. Results indicate that *E. brandti* feeds only on TOH, with greatly reduced feeding observed on corkwood, *Leitneria floridana* Chapman, and paradise tree, *Simarouba glauca* DC.

# Host use by the biological control agent *Longitarsus jacobaeae* among closely related plant species?

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The selection of representative test plant species for host-specificity testing is an important first step in pre-release studies of classical biological control projects. This may be a challenging task, particularly in projects where the target weed belongs to a species-rich genus. We present results from the host-range testing of a cold-adapted Swiss biotype of the flea beetle, *Longitarsus jacobaeae* L. (Chrysomelidae), a candidate for the biological control of tansy ragwort in Canada. Until recently, *L. jacobaeae* was considered to be monophagous under field conditions. We carried out adult feeding and oviposition as well as larval development bioassays with a large number of European and North American *Senecio* species to assess the fundamental host range of the Swiss biotype and to compare the results from the bioassays with the phylogeny, the plant secondary metabolite profiles and the physiology of the test plant species. The results will be discussed in the context of herbivore–plant interaction theories and of current recommendations for setting up test plant lists in biological control projects.

# Towards predicting establishment of *Longitarsus bethae*, root-feeding flea beetle introduced into South Africa for potential release against *Lantana camara*

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In an attempt to improve the selection of effective weed biocontrol agents, a study was conducted to demonstrate that the prospective agent, a root feeding flea beetle *Longitarsus bethae* (Coleoptera: Chrysomelidae), would establish by virtue of having the ability to cope with important environmental and ecological conditions in the release areas. Although *L. bethae* had been proven to be adequately host-specific to *L. camara* (lantana), releasing it into the environment would be a risk not worth taking unless there are reasonable grounds that it would establish and become prolific in its new range. A multi-factorial experiment was arranged to determine how four environmental factors (soil moisture, soil texture, the presence of *T. scrupulosa* and lantana variety) acting in combination might influence the survival of immature stages of this beetle. Soil moisture and clay content had the most substantial effect on survival of *L. bethae*, while the presence of *T. scrupulosa* and the type of lantana variety serving as a host had minimal influence on the beetles. Based on this investigation, a survival-prediction equation was derived. This was used to identify three geographic regions that are likely to be suitable, marginally suitable and unsuitable for *L. bethae* in South Africa. Based on host-specificity tests conducted previously and the results of the current study, it was strongly justified to release *L. bethae* in South Africa.

# Host-specificity testing the French broom psyllid *Arytinnis hakani* (Loginova)

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The broom psyllids are known to have strong co-evolutionary relationships with their related host plants. For this reason, the French broom psyllid, *Arytinnis hakani* (Loginova) (Homoptera: Psyllidae), was selected as potential biological control agent against its host plant, *Genista monspessulana* (L.) Johnson, a Mediterranean leguminous shrub invasive in Australia and California. Between 2002 and 2006, two types of host-specificity test were conducted on potted plants: (a) choice-without-target tests, which evaluated the capacity of the insect to lay eggs on test plant species in the absence of the natural host and (b) no-choice starvation tests, where the first-instar nymphs are forced to develop on test plant species other than the natural host. Over 92 species were tested in 47 genera covering ten plant families. The tests revealed that *A. hakani* can potentially develop on plant species from four genera within the Genisteae tribe (including the target), with nymphal development on species from two genera within the Thermopsidae tribe. The high number of species with nymph development in the genus *Lupinus* (16 of 25 tested) may lead us to reconsider *A. hakani* as a potential biological control agent against *G. monspessulana* in the USA. Further work on imported exotic lupines of economic importance to Australia is required to assess potential for release there.

# Prospects for the biocontrol of Banana Passionfruit in New Zealand with a *Septoria* leaf pathogen

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Seven closely related vine species of *Passiflora*, all with the common name banana passion fruit and of South American origin, have naturalized and become serious environmental weeds in various regions throughout New Zealand. Banana passion fruit is capable of smothering trees, particularly those at forest margins and in forest gaps. It often prevents regeneration of native plants and has therefore been classified as a priority weed for biocontrol by invasive plant biosecurity managers in New Zealand. It is also a significant environmental threat in Hawaii where it is known as banana poka. A successful classical biological weed control programme was undertaken with the release in 1996 of a virulent leaf pathogen, *Septoria passiflorae*. A similar biological control programme was initiated in New Zealand to explore the efficacy and safety of *S. passiflorae* for its potential introduction against this rapidly expanding and hybridizing weedy complex. Pathogenicity testing showed the fungus to be a virulent pathogen against the banana passion fruit weed complex, with promising biocontrol prospects. However, its release in New Zealand may be prevented due to its potential damage to the closely related commercially cultivated species *Passiflora edulis.*

# Novel preliminary host-specificity testing of *Endophyllum osteospermi* (Uredinales)

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*Chrysanthemoides monilifera* ssp. *monilifera* (boneseed), indigenous to South Africa, is a serious invader of native vegetation in southeastern Australia. The rust fungus, *Endophyllum osteospermi*, causes witches' brooms on bone seed in South Africa but has a long latent period, typically between 6 and 24 months between infection and the initiation of the witches' brooms. This long latent period makes the logistics of doing traditional host-specificity testing, in which all test plant species are inoculated and observed for symptom development, unfeasible for this rust fungus. Germination of aecidioid teliospores and penetration by basidiospores were observed on the surface of excised leaves of 36 test plant species at 4 days after inoculation and were compared to that on bone seed. Germinating aecidioid teliospores aborted on 14 test plant species, while no penetration was attempted on further 14 test plant species. Penetration only occurred, or was attempted, on eight of the 36 test plant species in addition to boneseed. Inoculating whole plants of nine selected test plant species confirmed the above results. Therefore, only the test plant species in which penetration occurred, or at least was attempted, need to undergo comprehensive host-specificity testing.

# Potential of *Ustilago sporoboli-indici* for biological control of five invasive *Sporobolus* grasses in Australia

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*Sporobolus pyramidalis*, *Sporobolus africanus*, *Sporobolus natalensis*, *Sporobolus fertilis* and *Sporobolus jacquemontii*, known collectively as the weedy sporobolus grasses, are exotic weeds causing serious economic loss in grazing areas along Australia's entire eastern coast. In one of the first attempts to provide biological control for a grass, a smut fungus, *Ustilago sporoboli-indici*, has been found to attack the leaves and flowering parts of *S. pyramidalis*, *S. africanus* and *S. natalensis* in South Africa. The potential of this pathogen as a classical biological control agent for all five weedy *Sporobolus* spp. found in Australia was evaluated in the glasshouse. The smut attacked *S. pyramidalis*, *S. africanus*, *S. natalensis* and *S. fertilis* but not the New World *S. jacquemontii*. Host range trials with ten native Australian *Sporobolus* spp. were also conducted. The extent of damage caused by the smut fungus to two weedy *Sporobolus* spp. (*S. fertilis* and *S. natalensis*) and two native Australian *Sporobolus* spp. (*S. creber* and *S. elongatus*) under glasshouse conditions was determined by measuring biomass and effects on flower and seed formation. The prospects for the smut as a biocontrol agent are assessed.

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# Theme 5:

# Regulations and Public Awareness

## Session Chair: Dick Shaw

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Keynote Presenter

# Regulation of biological weed control agents in Europe: results of the EU Policy Support Action REBECA

**R.-U. Ehlers**[1]

*Summary*

Biological weed control uses invertebrate biological control agents (IBCAs) as well as plant pathogens. Unlike North America, Australia and New Zealand, Europe has no central agency responsible for an environmental risk assessment (ERA) prior to the introduction of exotic IBCAs, and no classical biological control agents for weed control have been released in Europe. However, many exotic and native insect and mite species are currently used in European horticulture. Since the establishment in Europe of the exotic coccinelid *Harmonia axyridis* (Pallas) and concerns about potential displacement of indigenous coccinelids, proposals and guidelines for the regulation of IBCAs have been prepared. The European Union (EU) Policy Support Action Regulation for Biological Control Agents (REBECA) has contributed to the development and harmonization of guidelines and implementation of regulation procedures. However, regulation is organized on a national level. For progress in biological weed control, a well-recognized and knowledgeable European-wide organization dealing with the risk assessment and authorization of IBCA is urgently needed. Microbial weed control agents and products of natural origin have to be registered following the rules of the EU directive 91/414, which treats such biological control agents (BCAs) almost like synthetic chemical plant-protection products. REBECA has reviewed current legislation and guidance documents and made proposals for alternative, less bureaucratic and more efficient regulation procedures maintaining the same level of safety for human health and the environment but accelerating market access, increasing the availability of BCAs and lowering registration costs. Information on the progress of the REBECA Action is available on-line (www.rebeca-net.de). In Europe, the current expertise on risk assessment of biological weed control agents is limited to a few experts. A lack of knowledge at the level of regulation authorities will result in exaggerating risks and implementation of unnecessary regulation. It is therefore recommended to start a dialogue between weed control scientists and regulation experts immediately in order to prepare the ground for the use of biologicals in weed control in Europe.

***Keywords***: regulation, registration, data requirements, REBECA.

## Why regulation of biological control agents?

Regulation is implemented by governments when human activities cause or threaten to cause damage to the society. In order to avoid, prevent or minimize impacts, regulation is necessary. It should concentrate on safety aspects and try to minimize impacts on trade, economy and the environment.

Biological control agents (BCAs) for weeds, with very few exceptions, can be regarded as environmentally safe. In biological weed control, invertebrate agents including nematodes (IBCAs) and microbial pathogens have been released. These BCAs have been the most successful, cost effective, safe and environmentally friendly method of weed management. Nonetheless, our societies demand information on the risks and safety of BCAs.

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## Management of risks in modern societies

When regulating the use of BCAs, risks and benefits have to be carefully considered. Any kind of exaggeration of risks is causing trade-off effects (Graham and Wiener, 1995). Regulation might keep older, riskier technology (e.g. synthetic pesticides) in use. While policy and society demands a reduction of chemical control, over-regulation of biological control can result in a more widespread use of chemicals or increase damage caused by invasive weeds. In order to minimize trade-off effects, some fundamental rules of regulation should be followed. Prior to the development of regulation, a cost–benefit analysis should assess the magnitude of any problem and try to estimate the potential environmental damage. The result of the cost–benefit analysis will answer the question: Do benefits of regulation justify costs of regulation? A risk–trade-off-analysis should follow. Once trade-offs are identified in a quantitative and qualitative way, target risks and countervailing risks must be weighed and affected population (e.g. farmers vs endangered species) be estimated (Graham and Wiener, 1995). The last step is to develop effective and inexpensive tools. The search for cheaper and more effective tools to achieve the basic goal is of major importance and might produce creative solutions for risk assessment. These three principles are simple but also quite powerful. If they were taken seriously and implemented in the right way, they can improve risk regulation and potentially save money and damage to the environment. The analysis ensures that policy is driven by full appreciation of relevant risks and not by hysteria and alarm (Sunstein, 2002). Unfortunately, the implementation of regulation of BCAs is not always driven by this analytical approach but by the power play between interest groups and tradition. Rules, which have been developed in the past to protect the environment from synthetic chemical plant protection products, are now implemented on innovative, biological agriculture tools.

## The REBECA action

As set out in the European Union (EU) Community Agriculture Policy (CAP) package 2003, developing agricultural techniques, which are both ecologically sound and economically viable, will require new and powerful tools and assessment methods for the management of weeds, pests and diseases in European agriculture, horticulture and forestry. Biological control agents are part of these tools. However, despite considerable research efforts on biological and natural control agents (beneficial insect, mites and nematodes, microbial plant protection products, plant derived substances and semiochemicals), the number of such products on the market or projects in biological weed control in Europe is currently extremely low compared to other Organisation for Economic Co-operation and Development (OECD) countries. Therefore, the EU Commission published a call for proposals to organize a policy support action. As a result, the REBECA Action 'Regulation for Biological Control Agents' was started in January 2006. It reviews current legislation, guidelines and guidance documents at Member State and EU level and compares these with similar legislation in other countries, where the introduction of new BCAs has proven to be more successful than in the EU. The studies are available on the REBECA webpage. Based on specific input by researchers, regulators and product developers, proposals for appropriate and balanced risk assessment demands for BCAs have been developed. It is expected that no compromise to the level of safety will be made. In fact, more adapted risk assessment strategies might produce greater safety than occurs in the existing system. Proposals for a balanced regulatory environment will lead to better access to BCAs for growers and farmers and, therefore, to further reductions in the use of chemical pesticides. The results will serve as a scientific basis for reviewing current legislation and guidance for BCAs. The REBECA Action will last until December 2007 and will present and has discussed the outcome at a final conference in September 20–21, 2007 in Brussels. Results are also available on the REBECA webpage.

## Risks related with the use of IBCAs

No human activity is without potential risks. As a first step, REBECA analysed the real and potential risks related with the use of biological control agents. Risks related with IBCAs have been summarized by the REBECA Action (see results and presentations of REBECA Wageningen Workshop at www.rebeca-net.de): (1) Human and animal health: The probability of risks to humans is considered to be remote and limited to allergic reactions and bites and stings. (2) Plant and crop damage and development to nuisance: There are few reports of crop damage, e.g. *Macrolophus caliginosus* (Wagner) on tomato, or related problems such as the contamination of crop products, e.g. *Harmonia axyridis* (Pallas) in grapes in the USA. The latter can also be a nuisance when entering into houses. Plant damage is of less importance in the use of IBCAs to control plant pests; however, it is of major concern in biological weed control. (3) Environment: It was recognized that most important risks of IBCAs, real or perceived, are to the environment. These are: establishment of the exotic species in a new country, parasitism or predation of non-target species, competition or displacement of native species, perturbation of ecosystem functions, e.g. pollination, introduction of contaminating agents (pathogens, hyperparasites), and inter-breeding with native species.

## Data requirements for risk assessment of IBCAs

The REBECA Action considered all aspects of biological control applications, classical and augmentative (inundative) biological control as well as control of plant pests and weed control. Data needed to perform an environmental risk assessment (ERA) for IBCAs are limited to exotic species. For indigenous species, only data on identification should be required, and specimen should be deposited in recognized collections. For the ERA of exotic species, a hierarchical approach is recommended (Fig. 1).Within the scientific and regulatory expert groups in the REBECA Action, risks posed by exotic IBCAs were mainly related to host specificity, whereas impacts to health were considered negligible. For non-native species, additional safety data should inform about establishment, host range and dispersal. The hierarchical system proposed by van Lenteren *et al.* (2003), and updated in van Lenteren *et al.* (2006a, b) should be adopted (Fig. 1). Besides the significant reduction of weed populations, another major measure for success of a weed control release is the dispersal and establishment of a species. Consequently, dispersal and establishment are a pre-requisite for successful biological weed control, and ERA can therefore concentrate on the host-range testing. For host-range testing, REBECA experts recommended adoption of the testing scheme proposed by van Lenteren *et al.* (2006b) and to select non-target species for host-specificity testing as recommended by Kuhlmann *et al.* (2006), who proposed selection from three categories: (1) phylogenetically related; (2) occurs in the same ecological niche; (3) unrelated 'safeguard' species. Testing of the host range is first done in laboratory trials using one non-target species. If acceptance of non-target hosts is observed in no-choice tests, further tests need to include direct comparison of the acceptance and development on non-target species when the target species is simultaneously available. It is recommended that three treatments are compared with appropriate replication: (1) target species alone (control); (2) non-target alone (no-choice); (3) target and non-target together (choice). Often, these laboratory experiments provide false positive

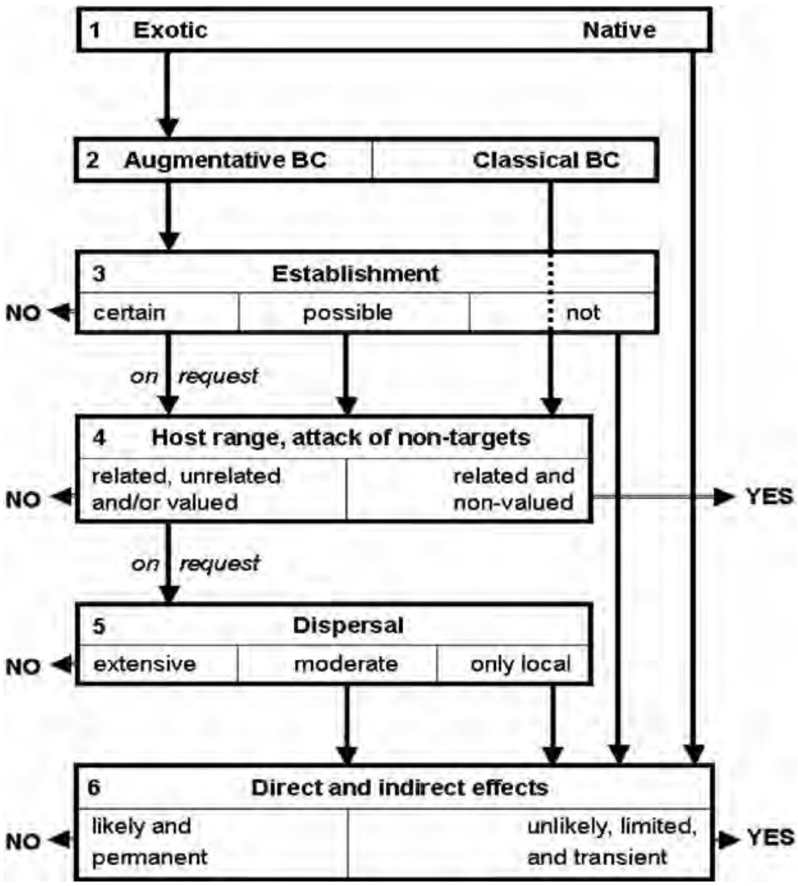


**Figure 1.** The hierarchical system proposed by van Lenteren *et al.* (2003), and updated in van Lenteren *et al.* (2006a, b) for risk assessment of invertebrate biocontrol agents. Exit from the system with the answer 'yes' will require a permit for release, and with 'no' the permit will be refused. Refusing might be particularly conflicting as the past use of exotic IBCAs for pest control has caused no or remote damage in Europe.

results on the host range. If the non-targets are accepted in these tests, experiments should be carried out under more natural conditions in contained environments such as large cages in the country of origin. Field observations on the host range in the ecosystem of origin might also be considered for a decision on the risks.

Direct and indirect effects are a summary of information gained from the available literature. When such information is not readily available, these effects may be estimated by 'expert knowledge' or generated from the data on establishment, host range and dispersal in the ERA. Examples of direct effects would include effects on non-target species and on other trophic levels (such as intra-guild predation and omnivory), hybridization and enrichment and vectoring. Indirect effects are those that occur when there is no direct interaction between the control agent and non-target species, such as competition and competitive displacement (see van Lenteren *et al*., 2003; Bigler *et al*., 2006). Indirect effects are difficult to quantify but are likely to be related to the scale of the direct effects. Risks related with the use of nematode are considered as exceptionally low as the dispersal is limited, and effects on non-target hosts are transient (Ehlers, 2003). However, this does not include weed control nematodes as they have not been considered yet. The REBECA Action has checked the applicability of its recommendations for both weed and pest control agents and recommends the use of both approaches. Methods on the procedures to assess environmental risks have been developed and widely discussed among experts (e.g. Bigler *et al*., 2006).

## How to regulate IBCAs in Europe?

What is not yet decided is how regulation of IBCAs should be implemented in Europe. Benefits by far out-weigh the risks posed by IBCAs. Therefore, these 'low-risk' products should not be over-regulated in order to enable the market excess also for small- and medium-sized enterprises or make possible cost-effective biological weed control programmes. The past has taught us that one of the major reasons why IBCAs have gained a market share of >150 million euros per year is the reduced level of regulation for these organisms. Of 90 nematode, mite and insect species in the list of biological control agents widely used commercially in Europe and neighbouring states, 40% originate from outside of Europe (EPPO, 2003). The European Plant Protection Agency (EPPO) had a 'Panel on Safe Use of Biological Control', which recognized that these species had been widely used in several EPPO countries and concluded that other EPPO countries may therefore presume with some confidence that these agents can be introduced and used safely. However, EPPO is not an organization which gives authorization for use of exotic IBCAs. Authorization in Europe is currently organized on a national level by different plant protection organizations, quarantine offices or agencies responsible for the natural environment. No European-wide organization exists which could authorize the use of IBCAs also for weed control. To minimize bureaucratic efforts and costs, the regulation of IBCAs should not be in the hands of organizations currently regulating agrochemicals and should follow rules specifically developed for IBCAs. REBECA has provided information on data requirements and forms for applications. What has not been solved is the problem of national regulation. As IBCAs cannot be restricted to national borders, a Europe-wide organization of the authorization is necessary. Committees including all stakeholders and member states should decide on the risks and produce consensus reports in order to agree on 'positive lists' of those IBCAs which pose no major risks to the environment. Similar approaches have been successful in the past (EPPO list). Such panels might be a solution for future assessment of risks related with the use of IBCAs in Europe and can also be implemented to assess the risk of IBCAs for biological weed control. However, such an organization must be recognized by all member states. Therefore, agreement between member states should be sought to identify one European organization which will be responsible for the risk assessment and authorization of IBCAs.

## Risks related with the use of microbial agents

The risks related with the use of microbial BCAs are pathogenicity to humans, mammals and other non-target organisms, effects of microbial secondary metabolites on non-targets, sensitization and allergic effects, genotoxicity, development of resistance to antibiotics, persistence in the environment, entering into or accumulation in the food chain, genetic instability, microbial contamination and other adverse effects on the environment. Most of the risks are reflected in data requirements for registration according to EU Directive 91/414 (Table 1).

## Data requirements and registration of microbial BCAs

The directive 91/414 does not make any difference between agents used to control plant pests or diseases or weed control micro-organisms. However, the registration process is expected to handle information requirements with some flexibility on a 'case-to-case' basis and not ask for data when no potential risk can be identified. Considering authorities follow these recommendations, many of the data requirements might not be relevant or applicable for weed control agents, and regulators might be able to waive data requirements, which would significantly ease the registration process for weed control micro-organisms.

**Table 1.** Data requirements for risk assessment according to Annex II B of the European Union Directive 91/414.

| Data requirements |
|---|
| Identity |
| Registration is on strain level, every new strain has to go through the process of risk assessment |
| Methods to identify and determine strain must be provided |
| Methods to identify impurities (contaminating micro-organisms, relevant metabolites) must be provided |
| Biological properties |
| Origin and natural occurrence |
| Target |
| Mode of action |
| Specificity |
| Dispersal and colonization ability |
| Genetic stability |
| Production of metabolites |
| Human health TIER I |
| Medical data |
| Sensitization (inhalation and skin) |
| Acute toxicity, pathogenecity and infectivity (oral, inhalation, intraperitoneal/subcutaneous) |
| Genotoxicity (metabolites of particular concern) |
| Short-term toxicity and pathogenecity (repeated exposure) |
| First aid measures |
| Human health TIER II |
| *In vivo* studies in somatic cells |
| *In vivo* studies in germ cells |
| Residues |
| Persistence and multiplication |
| Non viable residues |
| Viable residues |
| Fate and behaviour in the environment |
| Persistence and multiplication (soil, water, air) |
| Mobility |
| Effect on non-target organisms |
| Birds |
| Aquatic organisms (fish, algae) |
| Bees |
| Other arthropods |
| Earthworms |
| Non-target soil micro-organisms |

The procedure for EU registration of plant protection products containing micro-organisms is well defined. An applicant produces a dossier on the 'active ingredient' (ai) including all studies and data as summarized in Table 1. Pre-submission meetings of applicant and regulation authorities are recommended by the REBECA Action to discuss possible waivers and to prevent expensive investigations on non-relevant risks. The dossier is submitted to one national regulation authority of choice. This agency, the rapporteur member state, checks the completeness of the data set (completeness check) and then prepares a Draft Assessment Report. This report is sent to the EU SANCO (EU Directorate General for Health and Consumer Affairs) office responsible for the registration at the EU level, to the European Food Safety Agency (EFSA) and all member states. European authorities can consult experts on the risk of the ai. SANCO discusses the risk assessment with national regulation authorities. If the authorities consider the risks can be managed appropriately without major damage to users, consumers and the environment, then the ai is listed on the Annex 1 of Dir. 91/414. The final decision on the inclusion of the ai on the Annex 1 is taken by the Standing Committee on the Food Chain and Animal Health (SCFCAH) of EFSA together with SANCO (European Commission). If included, the ai cannot yet be used. National registration of the commercial product has to follow.

Data requirements of the formulated product involve trade name and composition (formulation), physical, chemical, technical properties (storage stability, particle size distribution etc.), data on application (intended use, mode of action, application method and rates, number and timing of applications, proposed instructions for use including safety indications), information on re-entry periods, cleaning of application equipment,

measures in case of an accident, efficacy data. Data on human health, exposure under the proposed conditions of use, residues, fate and behaviour in the environment and effect on non-targets are similarly requested as for inclusion on Annex 1. The procedure is laid down in the Dir. 91/414 which was amended by Dir. 2001/36/EC (Data Requirements Micro-Organisms). The European Parliament and Council are currently discussing a proposal for a regulation concerning the placing of plant protection products on the market, which will replace 91/414. All relevant information, guidelines, directives, position papers of stakeholders and results of the REBECA Action in relation to this discussion are available on the webpage.

## Are costs too high to allow use of microbial agents in weed control?

The situation with microbial BCAs is different. Due to the existing regulation, products do not make it to the market or are withdrawn immediately when regulation is required. Some member state governments try to find ways around 91/414. In Italy, until 2006, microbial products which only had the Latin scientific name as a product label did not need any registration. Switzerland had experts reviewing reduced data files. As a result, Switzerland has the largest number of BCA products available on the market. Now, the country wants to adapt to European rules. Some countries implemented a listing for low-risk products, such as plant growth promoters, and only demanded a minimum of safety data. Weed control agents might be possible candidates for such lists of low-risk agents. Within the EU, reduced data requirements for low-risk products are also discussed. However, it is not yet well defined what low-risk or basic products are.

Negative examples are also available for weed biological control. A European project considered fungal pathogens to control the Giant Hogweed but dropped the idea when participants learned that the agent had to go through registration according to 91/414 (M. Cook, 2007, personal communication). Whereas classical control programmes are area-wide approaches supported by public funds, the commercial application is using BCAs to substitute chemical measures on a limited area. In the past, the commercial use of weed control agents in Europe was restricted to The Netherlands, Germany and Belgium. Koppert (The Netherlands) sold the product Biochon containing the fungus *Chondrostereum purpureum* ([Pers.: ex Fr.] Pouzar) to control the introduced black prunus, *Prunus serotina* Ehrh. First introduced to diversify the forest, it later turned out to out-compete native shrubs and trees, and forestry wanted to remove the plant. The commercial success was limited. The product had been sold as a wood-rot promoter. When criticism arose and registration according to the directive 91/414 EEC was demanded, the company withdrew the product from the market. Costs related with the production of safety data would have outranged the possible commercial turnover by far (W. Ravensberg, 2007, personal communication).

Fortunately, regulation authorities now ask for less data and are prepared to give waivers for certain data requirements for microbial agents. Thus, costs for registration are lower than for chemical agents. However, one must calculate with approximately 0.4–3.0 million euros. In area-wide weed control, the costs related with the damage caused by invasive weeds might, however, justify costs for registration. Economic estimates in Germany concluded that the control of Giant Hogweed, *Heracleum mantegazzianum* Sommier and Levier, reaches a yearly amount of 12.3 million euros and of Japanese Knotweed, *Fallopia japonica* Houtt., 32.3 million euros. The costs to treat patients suffering from allergic asthma or rhinitis caused by *Ambrosia artemisiifolia* L. was estimated at 32.1 million euros (Reinhardt *et al*., 2003). However, these costs do not give indication of the market size for a commercial biological control product, particularly as for weed control cheaper chemical products are available and might be more effective when extinction during early establishment is required. But for reduction of problems with, e.g. the Japanese Knotweed, the introduction of pathogens could be a solution. The question is, of course, how to deal with registration. Following the European rules, pathogens would at least need to go through the registration procedure for inclusion in Annex 1.

Going through the list of requirements (Table 1), many questions could be answered immediately about risks of microbial weed control agents, and the conclusion would be ‘no risk’. However, SANCO rules follow the precaution principle, and authorities might possibly request experimental data for minor risks as well, which would make registration of a weed control agent an expensive exercise. Should, however, authorities give waivers for data on minor risks, then the registration might be less expensive than what has been spent in the past for the registration of BCAs to control insects and diseases. Scientific information and consideration of expert opinions can produce more confidence on the safety of weed control agents among authorities and justify provision of waivers.

Major concern with microbial agents is infectivity and pathogenicity to humans and possible effects of microbial metabolites. For weed control agents, these concerns might be of much less importance than for other BCAs. Medical doctors are well informed about every possible human pathogen or toxin producer causing food poisoning. EU Dir. 2000/54 gives precise information about which microbial organisms might cause damage to workers. Thus, for some organism, a risk assessment has already been done. Can we then waive the human

risk investigations if our microbial agent is not on the list? Can we argue that humans and other non-targets have co-evolved with these organisms and therefore authorities can waive many of the data requirements on the eco-toxicology? For instance, tests on earthworm toxicology are required, but a literature search has not resulted in any report of microbial pathogens found in these animals. Is it necessary to answer questions on residues possibly entering the food chain when the EU project 'Risk Assessment of Fungal Biological Control Agents' (www.rafbca.com) has provided evidence that fungal BCAs do not pose significant risks? Several requirements carried over from risk assessment of chemical products can certainly be waived. Should proposals of the REBECA Action be acceptable to policy makers and regulators, we might see a brighter future also in the use of microbial weed control agents.

To exploit the use of biological weed control agents in Europe, research projects should also consider the use of micro-organisms. Weed control scientists should contact experts experienced in the application for authorization of microbial control agents and together discuss relevant risks and data requirements and estimate costs related with the registration process. Public support might be available as no registration for a microbial biological weed control agent has ever been applied for before. Such an experience can afterwards serve as an example to define waivers and produce guidance documents to facilitate future registration procedures of microbial weed control agents. The scientific community dealing with biological weed control will not be able to progress without recognizing the information requirements by public authorities. Their request for data on the safety goes beyond the assessment of non-target effects on plants and the environment, which is identified as the major risk by weed control science. Contact between regulators and potential applicants should be made at an early stage of weed control projects to exchange knowledge and produce an environment for a scientifically based risk assessment, which considers concerns of all stakeholders. If weed control science and regulation will not enter into this dialogue, biological weed control in Europe will not have a future.

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# Avoiding tears before bedtime: how biological control researchers could undertake better dialogue with their communities

**L.M. Hayes, C. Horn and P.O.B. Lyver**

*Summary*

While many people support biological control of weeds, there are many others who doubt its safety and effectiveness, and universal agreement about what constitutes a weed is also rare. Scientists who attempt to develop biological control programmes without ensuring effective two-way communication with their communities may, at best, experience poor uptake of their research and, at worst, serious opposition that jeopardizes progress or even makes biological control programmes untenable. We describe a technique that allows researchers to engage in meaningful dialogue with their communities. People are brought together in an environment where it is safe to express opinions and concerns, where they are encouraged to listen and deepen their understanding, where they can discover common ground and build trust, and collectively find acceptable, or even novel, ways to move forward. This process could be adapted for other topics and social settings as long as key elements remain. We also describe some key messages about biological control of weeds that arose from this dialogue and suggestions for how things could be done differently in order to gain greater acceptance from the general public.

***Keywords***: communication, public engagement, decision making.

## Introduction

While many people are supportive of research to develop more natural and sustainable methods of weed control, others doubt the safety and effectiveness of biological control. Even other scientists are sometimes uncomfortable with or opposed to the practice (e.g. see Louda *et al*., 2003), and universal agreement about what constitutes a weed is rare (Stanley and Fowler, 2004). Currently, community perceptions are often more oriented to the threat biological control agents pose than the benefits of controlling weeds, and governments are becoming increasingly risk averse. These factors are causing extensive delays and fewer agent approvals in some countries while environmental damage caused by weeds continues unchecked - biological control as a discipline could be heading towards a crisis (Sheppard *et al*., 2003; McFadyen, 2004).

These challenges are not just restricted to biological control of weeds. Around the world, the presumption that science knows what is best for society is under challenge (Cribb, 2003), and scientists ignore public attitudes and values at their peril (House of Lords, 2000). Hipkins *et al*. (2002) found wide agreement (69%) to a statement that scientists should have to explain and justify their research to the public.

Lobbying for changes to biological control regulatory procedures is likely to only be a small part of the solution. Organizations undertaking biological control also need to ensure that effective two-way communication takes place between practitioners, regulators, critics and stakeholders to enhance understanding (Sheppard *et al*., 2003) and also to identify and resolve any potential areas of conflict or concerns or misconceptions at an early stage.

In New Zealand, and it would seem worldwide (Sheppard *et al*., 2003), biological control priorities are currently being decided and agents imported with little or no input from most stakeholder groups or the general public. Consultation is often undertaken only when

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it is a regulatory requirement and is not used to enhance the quality of decision making or improve public engagement. When human beings are suddenly presented with a well-developed plan, they will often oppose it because they feel backed into a corner.

## What is dialogue?

We use the term dialogue to describe two-way communication where each party listens to and respects the other point of view and seeks to find ways of moving forward that are of benefit to both parties. Increasingly, a number of dialogue processes are used worldwide for dealing with social issues, e.g. focus groups, citizens' juries, consensus conferences, deliberative polling etc.

Traditionally, the response to concerns about science and technology has been to try to better educate the public based on the presumption that if people understand the science better they will be more supportive. This assumes that science is universally comprehensible (Wilsdon and Willis, 2004), when the level of scientific literacy in the wider community is often not particularly high. In addition, this communication model misses the fact that science is not the only thing that people want taken into account during decision making. People often want to know about the need for the technology, the motivation behind developing it, the trustworthiness of the organizations undertaking it (Kass, 2001) and any potential unintended consequences in the long term. Even when people understand science better, they may not support projects if these conflict with their belief and value systems.

People assess risk in different ways. What the public may find acceptable may be at odds with objective risks as understood by science (House of Lords, 2000). Decisions about biological control agents have to be made on the basis of available information. Decision making must cope with the fact that the world is imperfectly understood, complex, dynamic and open-ended. Therefore, there can be major benefits in including people with a wide range of different expertises and perspectives in discussions and decision making processes to provide alternative perspectives that help to deal with inherent uncertainty (Kass, 2001). By ensuring that decision-makers listen to public values and concerns and give the public some assurance that their views are taken into account, the likelihood of decisions finding acceptance is increased.

## A novel approach to dialogue

This paper describes a novel dialogue approach that initially linked ideas taken from traditional Māori protocols and *The Seven Habits of Highly Effective People* (Covey, 1990) and was later pared down to key elements of both.

Māori are the indigenous people of New Zealand and have traditionally used marae (communal meeting area) to discuss or debate issues relevant to the community. Traditional practices and customs are used to welcome visitors to meetings and onto the grounds of the marae. During meetings, people commonly sit in a circular fashion with their backs to the inside wall of the wharenui (meeting house) often on mattresses on the floor. It is also common for the local people and visitors to sleep overnight in the meeting house if the meeting lasts for more than a day. Observing traditional Māori customs and values helps to foster a spirit of reverence amongst people and provides an alternate view of the world.

The '*Seven Habits*' offers ideas for living more effectively; some that we incorporated into our process included building trust, encouraging win–win thinking, achieving greater understanding through improved listening skills, valuing differences and creating 'third alternatives' (not your way or my way but a better way than any of us have thought of yet).

Initially, our process was used to undertake dialogue on the use of biological control for weeds and 1080 (sodium monofluoroacetate) to control mammalian pests (see Lyver *et al*., 2004; Hayes *et al*., 2004). Later, we tested a pared-down process on the development of a pest control strategy for the Hawke's Bay Region of New Zealand (see Lyver *et al*., 2006). In this paper, we focus mainly on the learning we gained from using our dialogue process.

# Methods

We tested our initial process four times between September 2003 and April 2004 at four different marae. We invited a wide range of stakeholders to participate including Māori, government departments, pest control agencies, the Environmental Risk Management Authority, industry groups, scientists, students and lobby groups. We aimed (with mixed success) to have a balanced gender and age mix. The number attending each event varied from 16 to 31. Where attendees were not local, we assisted with travel expenses. We also covered the cost of food and accommodation for all. Participants generally sat on mattresses on the floor in the meeting house, and most slept over on them too.

At the meetings held on marae, Māori facilitators helped participants to observe appropriate protocols. At all the meetings, an independent facilitator managed the group work. She established expectations, outlined the '*Seven Habits*', ensured ground rules were observed, managed any conflict, taught listening skills, assisted in the development of 'third alternatives' and reflected on the process. Main points of discussions were written down on large flipcharts, and a summary of the entire proceedings was produced.

On the first day, once the formalities and the expectations session were complete, our facilitator asked participants to present their points of view and encouraged everyone to listen carefully since they would have

to present someone else's viewpoint the following day (and they did not know whose this would be). She asked them to suspend judgement and assume they did not understand. She allowed questions of clarification only and stopped participants who challenged other's beliefs. At the end of the day, a social function allowed people to chat in a relaxed and informal setting.

On the second day, facilitators gave a demonstration of poor listening and good listening and then put participants into pairs (where possible with differing views). Each person had 30 min to explain their viewpoint again, and their partner was asked to reflect this information back to ensure they had clearly understood. At the end of the hour, the group came back together, and people took turns at presenting their partner's perspective. Afterwards, the group identified common ground and broke into smaller groups to think of 'third alternatives' before a final summing up and farewells. Participants completed an evaluation survey before leaving.

The pared-down process was used once in May 2005 and was designed to see if the process would work when real management outcomes were at stake and when the meeting was held at a venue other than a marae, in this case a winery. Chairs were arranged in a semi-circle, and there were some novel objects to create a sense of the unexpected, difference and novelty (e.g. deck chairs with cartoon characters on them, cush balls and an inflatable dog). People went home at the end of the day. Time that previously had been devoted to observing Māori protocols was spent working on outcomes. However, some key elements of Māori customs were still used but adapted such as making people feel welcome, introducing yourself fully and taking turns at speaking. Instead of reporting back other people's perspectives to the wider group, small groups revisited the task in hand based on their new understanding of each others' perspectives.

## Results

### Organizers' perspectives and observations

**Meeting preparation:** Setting up the dialogue meetings took much more time than expected (weeks rather than days). Many preliminary meetings, phone calls, emails and letters were required to identify potential participants and secure their participation. It was generally difficult to recruit stakeholders who were not already familiar with or involved with the issue. It was easier to get men to attend and present information than women. People from large organizations found it easier to attend than the self-employed. People were not always able to attend for the entire two days and, since the dialogue process builds upon itself, this was a disadvantage. Not all stakeholder groups were able to attend, and some had reservations about attending a meeting on a marae. There were no objections raised to attending a meeting at a winery.

**The actual events:** Overall, we felt that the meetings all worked well. The majority of participants showed enthusiasm for the idea of dialogue, and even those who came with some degree of doubt or suspicion participated fully without conflict under the guidance of our facilitators.

Sharing meals and, on the marae, the experience of communal living allowed people to get to know each other quickly. Social events at the end of the first day were an important and enjoyable opportunity for people to mingle and converse in a relaxed way. It was not necessary for people to stay together overnight for bonding to occur, but it did help. As people got to know each other and discovered common ground, it became more difficult to stop them from talking and come back to group work. Uncovering common ground was a vital part of the success of our dialogue process. For example, realizing they had a shared concern for the environment allowed participants to discuss contentious issues more constructively. We were pleasantly surprised at the amount of humour and camaraderie and the lack of aggression, particularly as some people attending (especially the 1080 meetings) had a history of prior conflict.

During the paired listening exercise, it was difficult for some participants to listen and reflect back, as it is not something they commonly have to do, and they needed help from the facilitators to stay on track. Some people expressed surprise at discovering something new about their partner's position. Often at the beginning of the exercise, people were surprised that a whole hour was set aside but at the end said they could have used longer. Overall, the presenting back of someone else's perspective to the wider group was done well and often involved humour. Some found the presenting-back useful because it allowed them to hear what people were saying a second time. Others realized how poorly we usually listen and how important good listening is.

### Participants' evaluations of the marae-based meetings

**Overall satisfaction ratings and written comments:** The majority of stakeholders rated their overall experience as 'great' or 'good' (Figure 1). All but one of the 81 participants said they would be prepared to participate in a similar dialogue process again. The most common reasons given were that it was an excellent non-confrontational forum to hear alternative views, share information, network with people and improve understanding and awareness. Some expressed surprise and delight that they had been able to participate meaningfully and talk to scientists. They were surprised to find that scientists were people like them with many of the same values. The scientists at these meetings were also enthusiastic about the interactions and to hear how others perceive their work and appreciated the sugges-

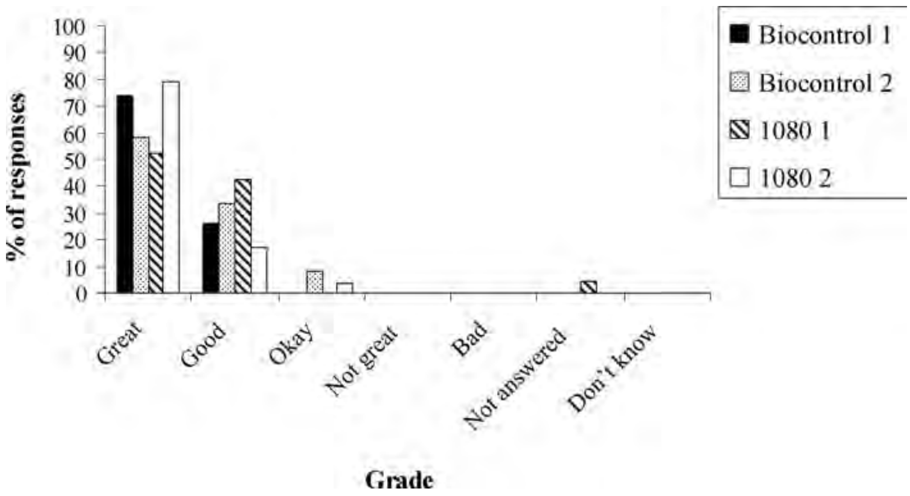


**Figure 1.** Stakeholder ratings of the overall dialogue meeting experience (biocontrol 1: *n* = 19, biocontrol 2: *n* = 12, 1080 1: *n* = 21, and 1080 2 *n* = 29).

tions for better ways of doing things that were generated (see Appendix 1).

**Long-term impressions:** We talked with a few participants some time after the event. Overall, people were positive about the effect that the meeting had had on them and on their networks. They had often maintained some contact with others they met at the meeting, even those who had previously viewed each other with some suspicion.

## Participants' evaluations of the pared-down process

**Overall satisfaction ratings and written comments:** Most of the 16 participants who filled in surveys rated the meeting as 'great' or 'good', and no one felt that it was 'not great' or 'bad'. Reasons why the meeting was worthwhile were similar to the marae-based meetings. Since many of this group had met before, it was noticed that some who are normally quiet spoke up and contributed in ways that they had not done before. Participants also commented that the process was fun and challenging, the venue was good, the meeting was well structured with different activities to keep people interested and focused, it was a safe place in which to exchange ideas, and useful outcomes were produced. The scientists involved were heartened to see that their work was valued and useful to others.

When asked about how they rated this style of meeting compared to other pest-management strategy meetings they had attended, there was a mixed response. Half said that the meeting was better than other meetings they had attended. A third did not know, and reasons for this were not explored, but some had not been involved in pest-management meetings before (Figure 2).

**Post-meeting follow-up:** We discussed how the meeting had gone with Hawke's Bay Regional Council staff the week after the event and again 5 months later. In

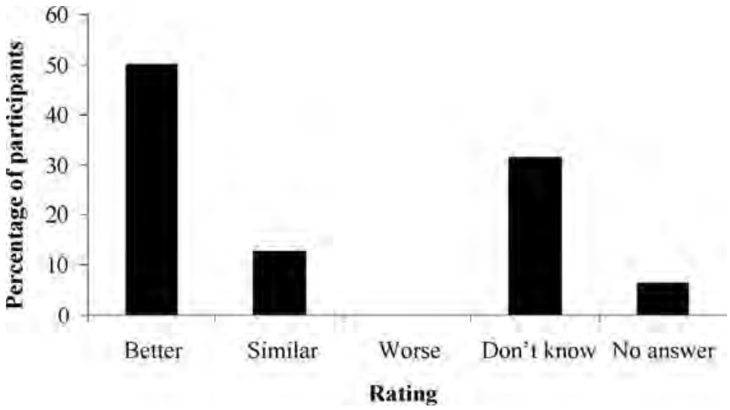


**Figure 2.** Participants' rating of dialogue meeting compared with other strategy development meetings (*n* = 16).

both sessions, they noted that the process had been enjoyable, that people attending had participated well and that it had been useful to expose people (including themselves) to a wider range of perspectives than usual. They also noted that, compared with other processes they had used, the dialogue process had worked well, as it got a lot more information and perspectives on the table before any debate was generated. This kept options open for much longer and provided a better overview of what was needed, wanted or expected. More was achieved in a shorter time. The process also created more ownership, buy-in, goodwill and enthusiasm. Involving scientists was considered to be beneficial as it allowed the group to learn more about science being undertaken on pest management and the ways in which science could strengthen the strategy. Overall, the dialogue meeting was deemed memorable as it was totally different from what people were used to, even though some had initially expressed some discomfort about coming to something without a traditional agenda.

## Discussion

Dialogue may seem to be a lengthy and costly process, but for potentially contentious issues, it could prove quicker and cheaper in the long run. There is some evidence that decision making involving appropriate public engagement is quicker and less controversial because more effort is put into framing problems, debating options and agreeing solutions than pushing relentlessly forward with unpopular decisions (Kass, 2001).

Winstanley *et al*. (2005) suggest that researchers benefit from discussions about social, cultural, ethical and spiritual issues associated with their research. Certainly all the scientists who participated in our dialogue events were surprised by just how useful they were in terms of clarifying understanding of issues, developing ideas for better ways of doing things and forming new relationships that would be helpful to them in the future.

There are many different ways to undertake dialogue, and inevitably, some topics and groups will be more challenging (Roper *et al*., 2004). Our process worked well regardless of the topic, so we believe it has wide applicability and could be adapted and simplified even further for use in different social settings as long as key elements are not lost. So what are these key elements?

### Initial relationship building

The dialogue event itself is very much the tip of the iceberg. The work required to develop relationships with stakeholders takes much more time than the event itself. Others trialling dialogue processes have also found this (Winstanley *et al*., 2005). It is easier to convince groups you have already had prior contact with and those with an established interest in the topic, to take part. People need to feel safe about participating, especially in situations where there has been a history of conflict. Some groups will find it more difficult to participate for reasons such as childcare or work commitments.

### Time required for dialogue

Adequate time is also needed to undertake a dialogue event. Participants ideally need to be present for the entire time because the process builds upon itself. Two days is a long time commitment, yet at the end many felt they would have liked more time. It may be possible to reduce the time needed by, for example, sending out information for people to read beforehand, but the same time pressures are likely to apply to any preparation. It may also have been easier to convince people to come to the meetings if they had been shorter in duration, and there will always be trade-offs between getting people to attend and what can be covered at the event.

### Facilitation

We found, like Cronin and Jackson (2004), that an important underlying principle is respect for others and their point of view. Therefore, it is important to have a skilled facilitator to run a dialogue event. Everyone taking part needs to be treated with equal respect, and ground rules (e.g. everyone gets a chance to speak without fear of interruption or being challenged) must be established early on. As some of our participants noted, the simple act of turn-taking and preventing interjections can have a very big effect on what gets ‘put on the table’ for later discussion. This, in turn, can have a major effect on the outcomes of that discussion.

Some stakeholders will be keen to get some sense of resolution from dialogue, which may or may not be the reason for undertaking it. It is important, therefore, to outline expectations at the start and formally close the dialogue at the end. It also needs to be clear upfront how any dialogue will contribute to decision making. It is important not to create any sideshows that distract from the dialogue. We found that the ‘*Seven Habits*’ became a distraction as people were interested in learning more about them, and it was better to just teach underlying principles.

### Active listening

Although we highlighted the importance of listening at an early stage, we found that most people had difficulty listening without interruption to points of view that challenged their assumptions or points of view. Other groups involved in dialogue have found the same (Winstanley *et al*., 2005). However, giving people the opportunity to ‘walk in someone else’s shoes’ for a

short time can be a profoundly useful way of improving understanding and goodwill.

### Enabling new behaviour

To be able to undertake good dialogue, people need to be able to step out of established or traditional expectations, roles and ways of doing things (Winstanley *et al*., 2005). The venue can help or hinder in this respect. A neutral venue with appropriate seating (Roper *et al*., 2004) where people can preferably sit face to face is best. A venue, such as a marae, that helps to break down normal patterns of behaviour and engenders respect and tolerance for difference, may be better than the usual meeting or conference rooms, which may have history or expectations attached to them. However, any room can be made better by paying attention to seating arrangements and having items that create a sense of novelty, difference and curiosity.

### Building relationships

The sharing of food is well known to be a good way of getting people to relax and engage with each other. Communal living can also help to break down barriers quickly but is not essential to good dialogue. Once the ice is broken and people start talking to each other, common ground is often found very quickly, which helps to build trust and allow people to work together constructively. It can be useful to explicitly identify this common ground because points of difference may be much smaller or even different from what people previously thought.

## Conclusions

Our process was a successful way of engaging stakeholders in dialogue about pest control. It was effective because it allowed each participant to weigh up the pros and cons for themselves in an environment where those in authority were not able to move into persuasion mode and where scientific knowledge was only one part of the equation. This process helped people discover common ground to learn new things about the standpoints of others and to build trust and understanding. It allowed constructive suggestions as to possibly more effective new ways of doing things and areas needing further thought or research. We believe that this process or some variation of it could be used to improve future decision making for all kinds of issues. Dialogue is not something to be entered into lightly, but the rewards can be enormous.

## Acknowledgements

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# Appendix: Feedback on biological control of weeds and 'third alternative' ideas

Overall, participants were supportive of biological control of weeds. They liked the idea of natural control but were not so keen that this involves introducing exotic organisms. Many were keen to minimize the use of chemicals. All want to have a choice of weed control tools, including new and better tools, and more information about integrated weed management.

Scientists need to communicate with their communities at a much earlier stage. There is no universal agreement about which plants are weeds, and people want to have more say in what targets are tackled for biological control. Some in the nursery industry would be prepared to sacrifice some of the plants they sell in order to control closely related weeds, and beekeeping is not necessarily compromised by biological control since the weeds do not disappear completely. There needs to be increased effort to improve public awareness of the seriousness of weeds.

Scientists need to have adequate funding to do communication, and many scientists would benefit from more training in communication skills - the public would prefer to hear from them than public relations people, and the media has its own separate agenda. People find terminology like 'biological control' a bit daunting, and it may be better to find more friendly descriptors.

A lot of dissatisfaction was expressed with traditional consultation processes. Undertaking communication only when it is time to apply to release a new agent is not satisfactory. Many stakeholders want to be involved in true dialogue. Scientists need to explore different points of view and concerns and take time to address them. People want cultural, spiritual and economic values and traditional knowledge to be taken into account, not just scientific values.

People want more information about all aspects of biological control, including expectations. Even if 100% guarantees cannot be made about effectiveness, people want 100% guarantees about safety. It will never be possible to do this, but many will be prepared to accept the risks once they have the opportunity to consider historical safety, the thoroughness of regulatory procedures and the discipline's commitment to best practice.

Follow-up must be done on all biological control agents, and scientists need to provide more assurances that this is occurring. There is a paucity of published studies worldwide showing what happens to weed populations after control of any kind. Obtaining sufficient resources to undertake adequate follow-up is always problematic, and scientists will need to undertake further dialogue with funders about this and find quicker and smarter ways of assessing impact.

Scientists need to tell the bad news as well as the good news and acknowledge negative aspects of biological control and any mistakes or failures (which we need to try to learn from). This will lead to more trust and buy-in. We need to find better ways of evaluating risk and dealing with uncertainty and make more of an effort to celebrate success so people know biological control can and does work and what the benefits are. Success should be defined at the start of projects.

# Field release of the rust fungus *Puccinia spegazzinii* to control *Mikania micrantha* in India: protocols and raising awareness

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*Summary*

Mikania weed, *Mikania micrantha* H.B.K., a perennial plant of neotropical origin, is a major threat to natural and plantation forests and agricultural systems in Asia and the Pacific. In India, it is a serious weed in the south-eastern and north-eastern states. The efficacy of herbicides to control mikania weed is short lived, and manual weeding is labour intensive and expensive. In this context, the rust fungus *Puccinia spegazzinii* de Toni, from Trinidad, shown to be highly specific and damaging to *Mikania*, was assessed for its control. Following a consultation process with the Ministry of Agriculture, Government of India and other local stakeholders, the rust was imported in 2004 into the quarantine facility at the National Bureau of Plant Genetic Resources in New Delhi. After additional host-specificity testing, field release was permitted by the Government of India in 2005. The rust was first released in tea gardens in Assam (north-east India) in October 2005 but did not establish, most likely due to the presence of a biotype of the weed that was partially resistant to the rust pathotype used. In Kerala (south-west India), releases of the rust were initially made in agricultural systems in August 2006, followed by forest sites. These releases are now considered to be successful; the rust has spread and is persisting. This is the first instance where a fungal pathogen has been used as a biocontrol agent against an invasive alien plant in continental Asia. An awareness-raising campaign on the merits of biological control of invasive alien weeds, targeting the general public, farmers, policy makers, forest officials and the scientific community, was undertaken. The range of methods, including engaging the media, publications and demonstrations are discussed.

***Keywords***: invasive alien species, plantation crops, classical biological control, Kerala, Assam.

## Introduction

*Mikania micrantha* H.B.K. (Asteraceae), a native of tropical and subtropical zones throughout the Americas, is a perennial, fast-growing invasive plant, capable of smothering agroforesty and natural forest ecosystems. It also invades many crops within home gardens and plantation production systems in the tropical moist forest zones of Asia and the Pacific (Waterhouse, 1994; Global Invasive Species Database, 2002). The preferred habitat for growth of mikania weed is open areas with moist soil. It occupies marginal lands, pastures, roadsides, uncultivated areas, degraded forests, plantations and agricultural systems. *M. micrantha* was introduced into the north-eastern part of India during the Second World War for camouflage of airfields and was later used as a ground cover for tea plantations (Parker, 1972). Thence, it has dramatically increased its range within India, spreading to over ten states especially in the north-east and south-west (Sankaran *et al*., 2001).

Mechanical control methods of mikania like sickle weeding, uprooting and digging are labour intensive, expensive and not effective in the longer term. Chemical control based on herbicides, such as glyphosate, and 2,4-D compounds, is practiced in several countries,

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but the efficacy is short term, and vigorous re-growth is observed after a few months of application (Sankaran and Pandalai, 2004). However, the weed was considered to be an ideal candidate for classical biological control using co-evolved natural enemies, since it is rarely a weed in its native range, where natural enemies limit its abundance (Cock *et al*., 2000).

Under a UK-Department for International Development (DfID)-funded project, fungal pathogens were assessed for their biological control potential of *M. micrantha* in India. No local pathogens were found to be suitable in India. However, the rust fungus *Puccinia spegazzinii* de Toni (Evans and Ellison, 2005) was selected from the broad range of coevolved fungal pathogens recorded from the neotropical, native range of the plant (Barreto and Evans, 1995), as a suitable candidate for introduction into India. This rust pathogen causes stem, petiole and leaf infections on *M. micrantha,* and 11 isolates from six countries were evaluated in the CABI Europe-UK quarantine glasshouse. The rust was found to demonstrate intra-species specificity, each pathotype infecting only a selected number of genotypes of its host (Ellison *et al*., 2004).

However, a pathotype from Trinidad (IMI 393067) proved to be virulent against a wide range of Indian populations of the weed, infecting all those tested from the Western Ghats, and hence was selected for further assessment. This pathotype was screened against 65 non-target species and found to be highly specific (infecting a limited number of species in the genus *Mikania*), damaging (infection often leading to plant death) and has a broad environmental tolerance (Ellison *et al*., 2008). After consultation with Indian stakeholders, permission was sought to import and release the pathogen in mikania weed-affected areas in the south-west (Kerala) and north-east (Assam) regions of India. This paper focuses on the processes involved in this and engagement with the public concerning this novel approach to weed control.

## Materials and methods

### Importation of *P. spegazzinii* into India

**Protocols:** India has a relatively long history of importing classical biological control agents for the control of invasive alien weeds; however, all the natural enemies have, thus far, been arthropods (Singh, 2001). *P. spegazzinii* was the first pathogen considered by the Indian authorities for classical biological weed control, and in fact, it was a first for mainland Asia. This necessitated consultations between biological control scientists, the Ministry of Agriculture, Government of India, the Indian Council of Agricultural Research, policy makers and other stakeholders to develop and refine the protocols. A dossier on the rust, produced by CABI Europe-UK for The Project Directorate for Biological Control, Bangalore (the nodal point for import of biological control agents into India), following the Food and Agriculture Organization (FAO) Code of Conduct (FAO, 1996; Ellison and Murphy, 2001), was submitted to the Indian Ministry of Agriculture. This document also included permission from the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago (where the rust isolate originated) for the use of their genetic resources, following the Convention on Biodiversity (http://www.biodiv.org/). Permission to import the rust into quarantine facility at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi was granted in September 2002.

*P. spegazzinii* can only survive in living plants; once the infected plant parts are dried, the teliospores are rendered non-viable. In addition, the firmly embedded teliospores do not readily survive scrapping from the host tissue. Thus, the rust had to be shipped to India on the living host plant. However, because high humidity in the shipment box would cause the embedded teliospores to sporulate (produce the infective basidiospore stage), the rust had to be shipped during the period post-inoculation but before the teliospores were fully viable (i.e. 2–10 days after inoculation). The shipment was hand-carried to avoid potential delays that could occur if it was sent as a cargo item. The rust was successfully established in the quarantine facility at the NBPGR in September 2004.

**Additional host specificity tests:** Following consultation with systematic botanists, mycologists and plant pathologists at Indian Council of Agricultural Research, an additional host-specificity testing list was drawn up, consisting of 74 test species/varieties of plants. Of these, 25 plants were closely related to the genus *Mikania* (members of Asteraceae), and the rest were economically important plants collected from different parts of India. The inoculation procedure is detailed in Evans and Ellison (2005) and assessment procedure in Ellison *et al.* (2004). For each test plant species or variety, eight replicate plants were used.

The screening was completed by April 2005 and a supplementary dossier (Kumar and Rabindra, 2005) was submitted to the Ministry of Agriculture with the application for field release of the rust. In June 2005, the Plant Protection Advisor to the Government of India from the Ministry of Agriculture gave the permit for release of *P. spegazzinii* in four identified areas, two each in Kerala and Assam.

Mikania plants inoculated with *P. spegazzinii* prepared in the quarantine facility of the National Bureau of Plant Genetic Resources were hand carried in polystyrene boxes to Assam Agricultural University in July and September 2005 and to Kerala Forest Research Institute in November 2005 and established in purpose-built facilities. Around 100 rust-infected plants were produced ready for field release at selected sites. The culture conditions under which the field inoculum plants were produced were found to be critical. Plants have to be pest free, therefore they are sprayed with

a general-purpose insecticide at least 1 week before inoculation; young, to ensure that they are growing at their maximum rate; and with abundant meristematic tissue since this is the most rust-susceptible part of the plant.

## Field release of *P. spegazzinii* in Assam and Kerala

The release strategy in Assam and Kerala (Table 1) involved placing large earthenware pots containing rust-infected *M. micrantha* plants in strategic positions in infestations of mikania weed. Positions were chosen in humid, slightly shady places, in a dense stand of the weed. Shoots of the plants in the surrounding vegetation were heavily sprayed with a fine mist of water before putting the rust-infected plants in position. Also, as much as possible, the shoots of the surrounding vegetation were pulled underneath the infected leaves, petioles and stems of the source plants. The release sites were regularly monitored, and progress of the rust infection recorded. Specific methodology at the different release sites is detailed below.

**Assam:** At site one, Experimental Garden for Plantation Crops (EGPC), the rust source plants were set in the ground by excavating a hole 20 cm in diameter and 30 cm deep for each pot, separated by at least 1 m, so the initial field infection could potentially be recorded separately for each inoculum pot. At site two, Cinnamara Tea Estate (CTE), the pots containing the rust-source plants were hung in a dense stand of mikania weed at the level of the tea table (flat top to rows of tea bushes where leaves are plucked) at about 75 cm height, in a shady place suspended from a bamboo pole with rope (Table 1). The mikania leaves were sparse at ground level due to shading by the tea bushes. All the release sites were sprayed with water twice a day for 15 days, except on rainy days.

**Kerala:** Each pot containing three rust-infected *M. micrantha* plants were placed on the soil surface within a defined 2 × 2 m quadrat separated from the next quadrat by 3 m; this potentially would allow the spread of each rust infection to be recorded separately for more than one generation. The total number of leaves, petioles and stems infected by the rust was determined for each site, at each date.

**Table 1.** Details of the *Puccinia spegazzinii* de Toni releases in the field in Assam and Kerala (India) during 2005–2006.

| Site | Site details | Release dates | Average temperature and relative humidity[a] | Inoculum source | Ecosystem type |
|---|---|---|---|---|---|
| **Assam** | | | | | |
| Site 1 | Experimental garden for plantation crops, Assam Agricultural University | a. Early October 2005<br>b. April 2006<br>c. June 2006 | a. 20–30°C, 85–90%<br>b. Not recorded<br>c. Not recorded | Six pots each release separated by at least 1 m | Mikania weed monoculture |
| Site 2 | Cinnamora Tea Estate | a. Early November 2005<br>b. April 2006<br>c. June 2006 | a. 20–30°C, 85–90%<br>b. Not recorded<br>c. Not recorded | Two groups of three pots separated by 4 m each release | Tea plantation (*Camellia sinensis* [L.] O. Kuntze) heavily infested with mikania weed |
| **Kerala** | | | | | |
| Site 1 | Echippara, Trichur Forest Division | a. 24 August 2006<br>b. 25 September 2006 | a. & b. 23.3–29.6°C, 70–100% | a. 11 pots<br>b. Three pots (10 m from release a.) | Agricultural system with mixed cropping of coconut (*Cocos nucifera* L.) and areca nut (*Areca catechu* L.). On the banks of a perennial stream with dense canopy |
| Site 2 | Palappilly, Thrissur Forest Division | 25 September 2006 | 23–28.7°C, 80–100% | Three pots | Degraded moist deciduous forest |
| Site 3 | Peechi, Thrissur Forest Division-Kerala Forest Research Institute (KFRI) campus | a. 19 September 2006<br>b. 30 October 2006 | See Table 3 | Six pots each release | Degraded moist deciduous forest |

[a] During 15 day inoculation period.

**Raising awareness:** An important part of the mikania weed classical biological control programme involved an awareness-raising campaign on the benefits of biological control amongst the communities where the rust was planned to be released, as well as the government policy makers, forest officials and scientists. At Kerala Forest Research Institute, the opinion of farmers concerning the use of host-specific natural enemies to control invasive alien weeds rather than chemicals was initially sought via farmer questionnaires and meetings. This was followed by demonstrations and exhibitions aimed at the agricultural and forestry extension services and students from universities and schools. A pre-rust-release workshop was held at Kerala Forest Research Institute and a post-release workshop in Assam Agricultural University to educate all stakeholders on the usefulness of biocontrol agents in controlling invasive weeds.

The media was also engaged: local newspapers in Kerala published articles on the release of the rust depicting the rust as a welcome solution to the weed problem; CABI Europe submitted press releases in the UK and India, resulting in popular articles being published in the press and radio interviews; Kerala Forest Research Institute in collaboration with the Audiovisual Research Centre, University of Calicut, produced two documentary films aimed at the general public. The first '*Weeds: the Biological Invaders*' was telecast all over India through the National Television Network. The second, focusing on biological control of weeds, is currently being edited prior to broadcast.

Publications have included a 'popular-style' book aimed at policy makers in the developing world; '*Invasive Alien Plants: Problems and Solutions*' (in press, CABI-Europe); and local-language brochure for Kerala farmers on the sustainable management of invasive alien weeds.

# Results and discussion

## Importation of *P. spegazzinii* into India

**Additional host specificity tests:** None of the 74 plant species inoculated was infected by *P. spegazzinii*, showing that the pathogen was highly host specific. Mild chlorotic flecks were observed on a few top leaves of four cultivars of sunflower, but the leaves recovered from the symptoms and there was no sporulation of the rust. The non-susceptibility of sunflower was also ascertained through histopathological studies (Ellison *et al*., 2008). All the inoculated sunflower plants showed normal growth and flowering, confirming the host specificity results from CABI Europe-UK and establishing that *P. spegazzinii* can safely be used as a classical biological control agent in India.

## Field release of *P. spegazzinii* in Assam and Kerala

**Assam:** At site one, the Experimental Garden for Plantation Crops, infection on field *M. micrantha* plants was observed 12 days after the release of the rust. Rust pustules were observed on leaves, petioles and stems of the surrounding mikania vegetation. However, the pustules were smaller than those normally observed on fully susceptible plants. The number of leaves infected ranged from 8 to 33, and maximum number of pustules developed on leaves ranged from 5 to 32. The number of stems and petioles infected was low (one to three). Following the first release, rainfall was continuous for 14 days, after which the common mikania leaf spot pathogen, *Cercospora mikaniacola* F. Stevens, became abundant on leaves. This sudden high level of *Cercospora* caused early senescence of the rust-affected leaves, hence slowing the spread of the rust infection. However, stem infections were not affected by *Cercospora*, so rust infection was able to progress in the field with the inoculum released from the infected stems. The disease progression was noted until January 2006, after which no progress was observed due to the non-conducive environmental conditions (high temperature and low humidity). Release of the fungus in April 2006 also resulted in good infection in the field, but the disease spread only a short distance from the inoculum source (30 cm in 9 weeks). Unfortunately, dry conditions following the June release prevented the rust from spreading.

At site 2, CTE, the rust infected the *M. micrantha* plants surrounding the inoculum source and spread further than at site one (1 m). As with site one, the pustules were small. However, progress of the rust infection was curtailed as the environmental conditions became non-conducive for natural spread of the rust. A similar result was found for the 2006 inoculations as at site one. The first inoculation at both sites was undertaken late in the wet season, when the conditions suitable for rust infection were already deteriorating. However, infection and spread was still achieved, but (at the chosen release sites) the rust was unable to survive the dry season. The 2006 inoculation did not lead to a significant level of infection and spread of the rust. This relatively disappointing result has been attributed mainly to the presence of a semi-resistant biotype of mikania weed present at the release sites, demonstrated by the small pustule size.

The evaluation of the pathogenicity of pathotypes of the rust against biotypes of the weed was reported by Ellison *et al*. (2004). This showed the presence of biotypes of mikania in Assam that were semi-resistant to the rust pathotype from Trinidad and that the Peruvian pathotype should be released in Assam as well. However, it was decided to proceed initially with importing the Trinidad pathotype into India, which was fully screened and is fully pathogenic to all biotypes tested

in Kerala (the region targeted during the first phase of the project, when original rust selection work was undertaken) and most of those from Assam.

**Kerala:** At all sites, the initial symptoms of the disease on the field *M. micrantha* plants were noticed a week after release of the rust. The results are summarized in Table 2. Similar results were observed at all plots inoculated in August and September although with varying degrees of disease severity and a maximum distance of spread of 1.5 m away from the rust-source plant. However, by October, the environmental conditions became non-conducive for spread of the rust, and levels of infection gradually declined (Table 3). Inoculations carried out in late October led to low levels of infection. By December, no rust infection was observed in the field.

The results, in general, indicate good spread of the rust from the source plant to field population of mikania weed in Kerala and Assam. Even though the field inoculations were carried out late in the wet season in Kerala, there was still also good spread within the field population until late October. Laboratory studies with the rust have shown that in the Western Ghats, optimum conditions conducive to rust infection (see 'Introduction') are likely to occur from June to September, although the temperature can go above optimum (up to 33°C) during August and September. However, beyond this period (until April or May), the maximum atmospheric temperature rises to over 40°C, and minimum relative humidity goes down to 30%. The conditions are more or less similar in Assam where the hotter conditions may extend until June. During this summer period, mikania weed tends to die back in open areas, although in areas with perennial standing water and along permanent streams, plants continue to grow and maintain leaves. Hence, over most of the mikania-infested areas, the rust will not be able to perpetuate. Evidence from the native range of *M. micrantha* and from glasshouse studies suggest that the rust will survive in living stems as cankers in open areas and on all aerial parts of plants surviving by permanent water. These 'rust refuges' could act as the inoculum source to initiate the rust epidemic as the rains begin and the mikania weed starts to reinvade.

## Raising awareness of the use of CBC

The result of a farmer survey showed that over 90% were willing to try the biocontrol agent in their farm. The Government of India adjudged the film '*Weeds: the Biological Invaders*' as the best documentary film on humanity, environment and human rights, in 2004.

Communication activities were undertaken to create awareness among the general public, forest officials, scientists and policy makers. The Tea Research Institute at Valparai, Tamil Nadu and plantation owners within Kerala approached KFRI on the possible use of the biocontrol agent to control mikania weed in their farms and plantations. Overall, this campaign showed that it is possible to cultivate a positive thinking on the use of biocontrol agent against invasive weeds in India.

# Conclusions

In Kerala in 2007, the aim is to significantly increase the frequency and quantity of inoculations of the rust

**Table 2.** Field infection of *Puccinia spegazzinii* de Toni on *Mikania micrantha* H.B.K. in Kerala, India.

| Progress of the disease in the field | Site 1 | | | | | | Site 2 | | Site 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dates of field release | 24 August 2006 | | | | 25 September 2006 | | 25 September 2006 | | 19 September 2006 | | 30 October 2006 | |
| Days after release | 25 | 54 | 75 | 100 | 22 | 40 | 20 | 70 | 20 | 37 | 17 | 36 |
| No. of leaves infected | 67 | 27 | 10 | 1 | 43 | 0 | 24 | 1 | 82 | 0 | 6 | 0 |
| No. of pustules per leaf | 1–3 | 1–2 | 1–2 | 1 | 1–3 | 0 | 1–2 | 1 | 1–10 | 0 | 1–5 | 0 |
| No. of petioles infected | 7 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 19 | 0 | 0 | 0 |
| No. of stems infected | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |

**Table 3.** Air temperature and relative humidity at Peechi (Kerala, India) during August–December 2006.

| Month | Air Temperature °C[a] | | Relative humidity %[a] | |
|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum |
| August | 21.7–24.8 (23.3) | 24.4–33.2 (29.6) | 52.0–95.7 (70.1) | 100 (100) |
| September | 22.0–25.1 (23.1) | 24.1–32.8 (28.7) | 60.0–98.7 (79.7) | 100 (100) |
| October | 22.1–25.8 (23.4) | 26.3–44.0 (35.8) | 38.9–62.6 (51.2) | 90–100 (99.3) |
| November | 22.1–25.1 (23.6) | 29.1–42.0 (37.7) | 45.0–59.0 (50.8) | 88–100 (99.0) |
| December | 18.3–25.4 (22.5) | 33.5–40.4 (36.6) | 31.3–54.6 (41.1) | 76–100 (88.6) |

[a] Mean value in parentheses.

in the field during the season most favourable to its spread (June to August), in order to build up the rust concentrations. As with the first releases in 2006, the selected areas will be those which encourage optimum rust propagation, e.g. cooler sites under shade or along the banks of perennial streams. It is suggested that once there is a critical concentration of the rust in an area, the infection will enter an epidemic phase.

Work is continuing in Assam to identify release sites with populations of mikania weed that are fully susceptible to the rust, where new releases can be made. In addition, the screening of the pathotype of *P. spegazzinii*, collected in Peru against a few selected plant species closely related to *M. micrantha* at CABI, has suggested that its selectivity, outside of its host species, is identical to the Trinidad strain. The Peruvian pathotype was subsequently (2006) imported into quarantine at National Bureau of Plant Genetic Resources, New Delhi, and additional confirmatory host-specificity screening is near completion. Permission to release this isolate in the field in Assam is being sought.

Awareness-raising activities will continue and will be combined with a rust-distribution programme by farmers and foresters, supported by the extension services, once optimum rust release strategies have been established.

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# What every biocontrol researcher should know about the public

**K.D. Warner,[1] J.N. McNeil[2] and C. Getz[3]**

*Summary*

Classical biological control is a public-interest science. This places a special responsibility on researchers and practitioners to communicate to the public about their activities and the benefits these provide to them. More than many other forms of science practice, biological control requires understanding public perceptions of their work and a coordinated effort to communicate with the public. Publicly addressing the risks of classical biological control introductions can foster a public consensus on appropriate risk-management strategies.

***Keywords***: public-interest science, risk perception, risk management, public outreach.

## Introduction

The practice of classical and conservation biological control is a public-interest science, done on behalf of the public and generally with public funds. The kind of knowledge produced by classical and conservation biological control work is of a public good character, meaning that it is non-rival and non-excludable; in other words, it is a pure common resource. Biological control research does not result in commodifiable knowledge (e.g. patents), and this trait distinguishes this form of scientific activity from many others. Consequently, as products of this science are not amenable to private property right protections, its practitioners - usually employees of public agencies or publicly funded universities - rely upon public funds to do their work. Thus, practitioners in the field of biological control have a special need to understand the public and cultivate public support for their work.

Declining public funding threatens to undermine the institutional capacity for biological control. The discovery of some nontarget effects has led some ecologists to assert that biological control is inherently risky and that much more precaution is necessary (Howarth, 1991; Lockwood, 1996). Thus, over the past two decades, critics, practitioners and regulators have publicly debated norms and policies that might apply to biological control. Several countries have implemented new regulations, prompting what some have described as an emerging regulatory crisis (Sheppard *et al*., 2003). Biological control researchers have long recognized the importance of cultivating public trust and support, which are critically necessary for policy support and public funding (van Lenteren, 2004, 2006). Members of the International Organization for Biological Control (IOBC) are advancing persuasive arguments that, while no pest-management strategy is risk free, biological control is often the safest and most cost-effective approach (van Lenteren, 2004; Delfosse, 2004, 2005). These kinds of initiatives are essential to sustain public funding and policy support for this scientific practice.

The relationships between scientists, scientific knowledge and the public are critical - yet contested - issues in the modern world and central to making progress toward a more sustainable relationship between humanity and the biosphere. In this paper, we report our research into the ways that public attitudes, public communication and public initiatives affect the practice of biological control. Many social scientists have analysed efforts to improve science communication and policy, and thus we begin by placing our work in this broader context. We will first review the obstacles and challenges illustrated by prior studies that underscore the importance of the public communication of science. We then report original data from field work in Canada and California on the relationship between

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several 'publics' and biological control efforts. This paper concludes with several recommendations to guide public initiatives on behalf of biological control.

## Communication, science and the publics

Scientists often avoid speaking to lay, public audiences and to the media, as their statements are often misquoted, taken out of context, clipped and distorted, sensationalized or even ridiculed (Hayes and Grossman, 2006). While these are valid reasons, our modern world consequently suffers greatly from the broad problem of scientific illiteracy.

However, the low 'public acceptance' of transgenic seeds/genetically modified organisms in the United Kingdom should serve as a cautionary case study for anyone with simplistic ideas about 'the problem' of 'public resistance' to a novel technology. Both the transnational corporations and government agencies involved in the development and regulation of transgenic organisms have behaved as though the public merely lacked information and that increasing the available knowledge would remedy the situation. During the 1990s, as more information was conveyed to the British public, public skepticism markedly increased. While the promoters of this technology did recognize the problem of scientific illiteracy, they proceeded to attack what they perceived as emotional responses, the public misperception of risk and irresponsible media coverage. One social scientist described their approach as an exercise in creating public alienation (Wynne, 2001).

Regardless of what one thinks of these technologies, the public outreach strategy for genetically modified organisms (GMOs) in the UK was a disaster. Many science communication scholars could have predicted this outcome because prior research in this field has consistently revealed that the critical issue is not scientific illiteracy of the public but rather the uneven levels of public trust in expert scientists and the political regulatory institutions (Rampton and Stauber, 2002). Studies have demonstrated that more knowledge communicated to the public about a controversial scientific practice can very easily result in amplifying rather than diminishing public fears (Gregory and Miller, 1998). At least in advanced industrial societies, most people generally trust, and thus filter out, scientific knowledge and technologies as a matter of routine. Querying the general public about their opinions regarding a scientific practice about which they know little may provide them with specific information that they find disturbing. Consequently, while science practitioners may use new findings to increase the level of public knowledge, building trust may be a more effective strategy. Therefore, any effort to communicate expert knowledge to the public must also seriously address the need to establish credibility and foster trust. The interdisciplinary field of Science, Technology and Society (STS) has addressed many of these kinds of questions for decades (Gregory and Miller, 1998).

## What do we know about what the public thinks about biocontrol?

In North America, two social science surveys have assessed public knowledge about biological control, one in California and the other in Canada. The ash whitefly, *Siphonius phillyrea* (Haliday) (Homoptera: Aleyrodidae) was introduced into California's urban landscape, causing millions of dollars of damage through defoliation. The California Department of Food and Agriculture's Biological Control Program introduced a parasitoid, *Encarsia ianaron* (Walker) (Hymenoptera: Aphelinidae) which established and provided a highly successful control effort (Pickett *et al*., 1996) that provided between 219 and 298 million US dollars in benefits to the public. However, shortly afterward, the budget of California's overall Biological Control Program was cut, resulting in half of the permanent scientific staff being let go. This program was vulnerable to such vagaries of California State funding because it did not have a dedicated revenue stream, nor did it have sufficiently powerful political allies.

To assess potential public support for such a revenue stream, Jetter and Paine (2004) surveyed consumers about their economic preferences for three strategies (chemical pesticide, biorational insecticide or introduced natural enemy) in controlling an invasive pest of urban forest landscapes. They provided respondents a booklet with background information on these pest-management options and asked urban homeowners to report their relative willingness to pay for them. The findings suggested that social and financial support by urban residents could be tapped to fund the introduction of classical biological control agents for landscape pests.

Under the auspices of the Canadian Biological Control Network, several Canadian researchers conducted a Canada-wide telephone survey in 2005 to determine public perception of biological control as an alternative to the use of traditional pesticides and GMOs (McNeil, personal communication). Here the thrust was to assess the perceived risks of these three pest-management options. Although the data from this survey are still being analysed, initial findings indicate that Canadians generally consider biological control to be safer than conventional agrochemical pesticides in agriculture. The findings also indicate that Canadians, especially those of middle age, would like more information about pest-management strategies used in their food production, a finding consistent with other surveys about how much information consumers would like about the conditions of their food production (Eilenberg and Hokkanen, 2006).

A fundamental limitation of these types of surveys is that they assess public opinion, not actual behavior. Neither of these surveys query who among the public would actively participate in deliberations about the importation of biological control agents or funding for such initiatives if the opportunity arose. However, in the USA and Canada, there is no formal process for soliciting public input into biological control importation decisions (Mason *et al*., 2005). Thus, other participants in the administrative policy process must represent the public interest.

## Identifying clients for biological control

Warner and Getz have been conducting a study since 2004 of the social and economic factors in California serving as obstacles and opportunities for further implementing biological control in agriculture. Our work reveals the critical role of two institutions that recognize the value of biological control: county agricultural commissioners and growers' groups. Agricultural commissioners are appointed by locally elected county officials, and thus they are responsive to local residents. Commodity board research directors are hired by organizations of farmers, and they understand their role to be supporting the kind of research that will serve the needs of their farmers. Interviews indicate that both play a critical intermediary role for county residents/taxpayers and large groups of growers, which are two groups interested in pest management. The dynamic works like this: when an urban landowner or a farmer of a specific commodity has a problem with a new pest, s/he contacts the agricultural commissioner or commodity board research director, who, in turn, contacts a university specialist or state program researcher. The commissioners and research directors function as key intermediaries, or knowledge brokers, from land managers to the knowledgeable expert, who then, if appropriate, conduct research and share information in turn with these intermediaries. This arrangement works well, as long as the research institutions are properly funded, but as the section above narrates, this is not presently the case. Scientific knowledge is circulating through this system, but because political knowledge about the benefits does not, financial resources are erratic.

The evolution of environmental regulations in California has made conventional insecticides increasingly more difficult to use. Consequently, both urban landowners and farmers are generally open to considering biological control if it proves effective and affordable. However, even these groups, who are able to identify the value of university and state biocontrol programs, know very little of the budgetary constraints faced by the scientists carrying out the research. An opinion survey of land owners and farmers would likely indicate support for these researchers (and any other expert knowledge that could help them) but does not automatically follow that this public's interest will be transmitted to government funders.

Our interviews with agricultural commissioners and commodity board research directors indicate that they are quite aware of, and concerned about, the diminishing institutional capacity of the University of California and the state Biological Control Program. Their professional responsibilities include helping (urban and agricultural) land managers control pests and ensure they conform to environmental regulations. Research directors are particularly concerned that the number of scientists conducting practical research in biological control has declined significantly over the past few decades. One noted that she could provide funding for any genuine biological control proposal that had the potential to advance knowledge of that crop's farming systems but that the number of researchers in the field has diminished significantly. Another, representing a major crop in the state, said that there was only one scientist in California that could help him with one of his major pests.

The agricultural commissioners who are often the first officials to receive a phone call from a distressed landowner have legislatively mandated responsibilities for protecting their county from noxious weeds and insects and also for enforcing state pesticide laws. Thus, they too experience the tension of having to coordinate pest-management efforts but within the limitation of existing laws. Consequently, they are among the most active consumers of the research knowledge and the biological control agents provided by California Department of Food and Agriculture (CDFA)'s Biological Control Program. They advocate for funding this program, but they are somewhat constrained as their own county activities depend on the State Secretary of Agriculture for funding.

One particularly noteworthy institutional vehicle for building public support for funding has been the California Weed Management Area Support Program (California Department of Food and Agriculture, 2006). With relatively modest state funding, this program has fostered local networks of concerned landowners and agencies to focus attention on noxious weeds. It has leveraged US $5.4 million of state money to attract over US $7 million of additional funds, but more important has been its ability to provide a vehicle for local landowners to coordinate their efforts and educate the public. Weed-management areas (WMAs) provide the social infrastructure to cooperate in a meaningful way with the state's Biological Control Program. The current WMAs rest on a long history of coordinated pest management in California (Baker, 1988; Warner, 2007). They are essential for coordinating widely dispersed weed-management activities but also for activating existing social networks to advocate for continued funding. Members of the public who have benefited from coordinated weed eradication are much more likely

to provide the political support for biological control. One landowner who has benefited by such a program is much more likely to take action in support of biological control than one million consumers who express a favorable opinion on a mass survey.

## Public initiatives on behalf of biological control

From the observations above, it seems that biocontrol is a terrific pest-management strategy but that it faces worrying trends. Science funding and policy in the industrial countries now operate in a new, more challenging political context. No scientists can count on stable funding or policy work. This is particularly true of a public-interest science and one that must now confront the 'controversies' of non-target impacts. We therefore recommend that the biocontrol community develop strategic alliances with several potential 'publics'. Developing coordinated science communication policy requires more work but few financial resources.

Biological control has five publics, with different communication needs: client communities (land managers and farmers); funders (interested in pest management and invasive species); regulators; potential scientific allies (public-interest ecologists interested in pest-management alternatives); and contrarians (scientists who are philosophically opposed to biological control). Communicating with these five requires a carefully targeted message. An example of this kind of linking of research and public communication can be found in the Ecological Society of America's 'Sustainable Biosphere Initiative' (SBI) presented by Lubchenco *et al*. (1991), described by Lubchenco (1998) and analysed by FitzSimmons (2004). The SBI has developed research briefs for policy makers through their 'Issues In Ecology' publications, and this kind of effort could be copied by the biocontrol community. A 'biocontrol science communication working group', perhaps coordinated by the IOBC, could engage policy makers by presenting the value that this science could make to managing invasive species and pests.

## Conclusions

Conclusions and recommendations in this paper include:

1. The public-interest character of biological control requires ongoing initiatives to cultivate public and governmental support. The work of IOBC global and regional sections is to be commended. We recommend that IOBC cultivate the help of social scientists, especially those who study STS and science communication, to strategize a coordinated and sustained effort to engage the public in its many forms.
2. Identify and partner with institutions most likely to benefit from your work, and encourage them to represent the public value of biological control in the public sphere. The commodity organizations and WMAs are excellent examples of how this kind of partnering can bear fruit.
3. Strategic analysis suggests that favouring outreach efforts which cultivate partners and clients who can serve as credible messengers to public policy makers and funders is likely to be more fruitful than efforts to outreach to generic public 'masses'.
4. Recognize that most publics evaluate your work not on the basis of scientific knowledge or its merit but rather on trust. Build trust through collaboration with credible partners, and be transparent about risks, risk-management efforts, peer review and the value of client participation in this work.
5. Do this as a group, through institutions and networks, to justify biological control for its public good features (not because of original scientific discovery). Emphasizing the public good features of biocontrol can convert passive acceptance into active advocates for funding and supportive policy. Partners can serve as credible messengers, conferring legitimacy to your work and increasing the likelihood of funding for providing pesticide alternatives and strategies for controlling invasive species.

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# Is the 'Code of Best Practices' helping to make biological control of weeds less risky?

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Although practitioners know that biological control is one of the safest approaches to managing invasive species, they also realize that this tool must be used wisely and appropriately. The sub-discipline of biological control of weeds was the first to acknowledge the need for universal standards to both guide those practicing weed biocontrol and to allow outside observers to better discriminate 'good' biological control of weed practices from those that are ill-advised. In 1999, at the close of the Xth Biological Control of Weeds Symposium in Bozeman, Montana, the delegates overwhelmingly adopted a resolution requesting that practitioners adhere to the 12 guidelines of the 'Code'. We review the 12 guidelines and how they apply to both groups that are involved in biological control: (1) the scientists performing the research necessary to find, release and establish the biocontrol agents and (2) those who re-distribute established agents. We also discuss the results of a recent survey we conducted to assess the impact the 'Code' was having on biological control of weeds in north-western USA. Finally, we provide some examples of how the 'Code' has been implemented by various agencies in this region and ponder on why it seems to be having less impact elsewhere.

# The new quarantine facility, St. Paul, MN, USA

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The University of Minnesota Agricultural Experiment Station and the Minnesota Department of Agriculture combined resources to construct a state-of-the-art quarantine facility, which began operation in 2003. Drs David Ragsdale, University of Minnesota, and Dharma Sreenivasam, Minnesota Department of Agriculture, were instrumental in obtaining the funds and commitment for this biosafety level 2 (BL2) facility. BL3 capabilities are being added with potential initial targets including Asian soybean rust (*Phakopsora pachyrhizi*) research. The first invasive plant biological control effort at this facility is an ongoing cooperative effort with Centre for Agriculture and Biosciences, International (CABI) Bioscience, Delémont, Switzerland, to complete host-specificity screening for *Ceutorhynchus* spp. for biological control of garlic mustard (*Alliaria petiolata*). Current research at this facility involves studies in taxonomy, genetics, life history, host specificity, behavior, control efficacy, experimental release and post-release evaluation of plant and insect biological control agents.

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# Biological control of weeds at the USDA-ARS-SABCL in Argentina: history and current program

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The South American Biological Control Laboratory (SABCL) has a long and successful history in the biological control of weeds. Since its establishment in Argentina in 1962, the SABCL has worked with 29 target weeds and more than 110 biocontrol candidates; 15 were field released in many countries around the world, while nine are still in quarantine for further testing. Most of the weeds investigated at SABCL are invasive species in the USA, Australia, South Africa and other countries. The first SABCL projects were alligator weed (*Alternanthera philoxeroides*) and water hyacinth (*Eichhornia crassipes*). A total of 13 natural enemies were studied against these two weeds from 1962 to 1980, nine of which were field released. Currently, 56% of the SABCL scientific staff ($n$ = 16) is assigned to weed research on the following targets: water hyacinth, alligator weed, fanwort (*Cabomba* spp.), Brazilian peppertree (*Schinus terebenthifolius*), balloon vine (*Cardiospermum grandiflorum*), pompom weed (*Campuloclinium macrocephalum*), Barbados gooseberry (*Pereskia aculeata*), Brazilian waterweed (*Egeria densa*), water primrose (*Ludwigia hexapetala*) and Lippia (*Phyla canescens*). Research on this weed program is funded by the US Department of Agriculture, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, and the Plant Protection Research Institute (PPRI), South Africa.

# A quarter of a century of contributions from the FDWSRU in biological control of weeds

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Evaluation of foreign plant pathogens for biological control of weeds was initiated at the United States Department of Agriculture, Agricultural Research Service (ARS) in the mid 1970s. Justification for locating this research effort at the Foreign Disease-Weed Science Research Unit (FDWSRU), Ft. Detrick, is a containment greenhouse facility that enables evaluation of exotic pathogens of crop plants and weeds. Since transfer to ARS, three foreign weed pathogens evaluated in containment have been introduced into the USA under permit from federal and state regulatory organizations. These pathogens, all rust fungi, are: *Puccinia chondrillina*, *Puccinia carduorum* and *Puccinia jaceae* var. *solstitialis*. The program at FDWSRU has since expanded to include 2.5 research scientists with full technical support. A number of new projects have been initiated, including rust fungi and facultative saprophytes on *Salsola tragus* (two pathogens), *Acroptilon repens* (two pathogens) and *Crupina vulgaris* (two pathogens). A new thrust into the use of floral smut fungi on *Silybum marianum* and *Carduus* thistles is being pursued as well. Several new pathogens also have been discovered in Greece, Hungary, Russia, Tunisia, Turkey and the USA. This paper is a review of developments, accomplishments and current and anticipated research at the FDWSRU.

# Protocol for projects on classical biological control of weeds with insects

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A protocol for projects on classical biological control with insects was developed by European Biological Control Laboratory (EBCL) staff to help biocontrol workers and biocontrol consumers. The general problem of finding, evaluating and eventually introducing a biological control agent is a complex process formed by unequal parts: politics, administration and science. All process includes eight phases where several agencies at different levels, federal, state and University, are involved. First phase involves information on targets; second phase, selection of potential agents; third phase, overseas explorations, collections and research; fourth phase, importation into USA for further study and evaluation; fifth phase, release and establishment in the USA and elsewhere; sixth phase, evaluation in USA of agents released involving State, University Region, and County authority; seventh phase, evaluation in USA, ARS State agencies methodology of development and rearing of selected agents to be released; and eighth phase, evaluation study in USA ARS State biological and ecological aspects such as biology, economic impact and competition study.

# Weed biological control evaluation process in the United States - past and present

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Starting in 1957, the United States Department of Agriculture (USDA) implemented outside agency reviews to evaluate the introduction of weed biological control agents. A group of five agencies were identified as the Subcommittee on Biological Control of Weeds and ascertained if targeted plants were weeds and identified non-target test plants that should be examined for host specificity. During the 1960s, the group expanded and began to exchange information between the USA and Canada. In 1971, the subcommittee became the Working Group on Biological Control of Weeds (WGBCW) with the addition of four agencies. Also, information exchange began with Mexico. In 1987, the group was revised, becoming the Technical Advisory Group (TAG) of Biological Control Agents of Weeds. It is currently composed of 17 organizations and Canada and Mexico officials and provides guidance and recommendations to researchers and regulators. From 1987 through 2005, the TAG reviewed 153 petitions, with 105 petitions requesting the release of biological control agents. Recent examinations have identified that 57% of the first-release petitions received a favourable recommendation. Through the re-evaluation process, eventually over 75% of the agents that were initially requested for release received favourable release recommendations.

# Biocontrol capacity of ARS research group in Central Asia and surrounding areas

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The Kazakhstan biocontrol research group was organized as an ARS cooperator in 1994. Now, the station consists of five entomologists, two botanists, one soil scientist, one Geographic Information System (GIS) specialist and several technicians and equipped by field and laboratory equipment. The capacity of biocontrol research is based on the native distribution of many Central Asian plants that are weeds in western USA and Canada: 36 weed species are native to Central Asia such as perennial pepperweed (*Lepidium latifolium*), Russian thistle (*Salsola* spp.), Russian knapweed (*Acroptilon repens*), yellow starthistle (*Centaurea solstitialis*), medusahead (*Taeniatherum caput - medusae*) and other weeds, as well as for several serious introduced insect pests. The Almaty, Kazakhstan station is well situated to conduct explorations for control agents for many weeds. The close proximity of these weeds to the Almaty station allows for inexpensive, season-long studies of field ecology, behaviour, host-range observations in the field, and no-cage formal testing which cannot be done in the USA and which provide the most realistic evaluation of these critical factors. Good relations between Kazakhstan and Russia and other Central Asian countries and a common language (Russian) and cultural similarities allows open travel and free scientific exchanges with these countries unavailable to most western scientists.

# USDA-ARS Australian Biological Control Laboratory

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The staff of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Australian Biological Control Laboratory (ABCL) actively search the natural areas of Australia and Southeast Asia for insects and other organisms that feed on pest insects and plant species that are invasive in the USA. Based in Brisbane, Queensland, the ABCL is operated by the USDA-ARS Office of International Research Programs (OIRP) through a cooperative agreement with the Commonwealth Scientific and Industrial Research Organization (CSIRO). Many invasive weeds in the USA such as the broad-leaved paperbark tree, *Melaleuca quinquenervia*; Old World climbing fern, *Lygodium microphyllum*; hydrilla, *Hydrilla verticillata*; and Australian pine, *Casuarina* spp. are native to Australia. However, the native range of many of the weed species continues northward into tropical and subtropical Southeast Asia. With excellent collaborators in this region, ABCL has the capability to find the most promising biological control agents. Research conducted at ABCL includes determination of the native distribution of a weed species, exploration for natural enemies, molecular typing of herbivores, ecology of the agents and their weed hosts, field host-range surveys and ultimately preliminary host-range screening of candidate agents. In collaboration with US-based ARS scientists, agents are selected for further quarantine studies and possible release in the USA.

# Status of biological control in Australia, policy and regulatory influences

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Currently, biological control of weeds in Australia is experiencing a reduction in the number of newly nominated targets and a reduction in the number of agents released from ten per year a decade ago to two per year since 2000. At the same time, there has been a reduction in scientific activity as measured by publications and loss and non-replacement of experienced staff specializing in biological control. This is despite increasing recognition of the threats posed by invasive weeds where biological control is often the only suitable long-term solution. There are, however, indications that the situation is improving. This presentation will examine the status of biological control in Australia and the influence of legislation, regulation, infrastructure, national committees and funding policy on future developments. The future of biological control will also be examined in the context of a developing environment of acceptance of alien invasive species (new ecosystems).

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# Theme 6:

# Evolutionary Processes

## Session Chair: Ruth Hufbauer

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Keynote Presenter

# The primacy of evolution in biological control

G. Roderick[1] and M. Navajas[2]

*Summary*

Evolutionary biology underlies much of the theory and practice of classical biological control; yet, its importance remains largely unappreciated. Procedures of classical biological control, including agent selection, quarantine, pre- and post-release studies, establishment, agent–target interactions, non-target effects and even risk analysis, all involve, to a greater or lesser extent, evolutionary issues and all can likely be improved by a better understanding of evolutionary processes. In this paper, we examine these processes, particularly adaptation and genetic variability. We also point to promising emerging areas in evolutionary biology, population genetics and related fields that can better inform biological control. These include DNA barcoding, whole genome sequencing, distributed databases and new computational approaches. Despite their promise, evolutionary studies in the context of biological control can be difficult: Evolutionary studies typically are studied over multiple generations and often the ideal experimental protocols are logistically complex. To address these problems, we draw parallels to invasion biology and emphasize the need for long-term, follow-up studies, even when biological control is not successful.

***Keywords***: adaptation, genetic variation, evolution, selection.

## Introduction

A central assumption of classical biological control is that predators, parasites, pathogens and herbivores will maintain their affinity for the target pest(s) while adapting to exploit their new habitats (see Simberloff and Stiling, 1996). Yet, adapting to non-target hosts is not desirable and frequently not acceptable. Adaptation is an evolutionary process, and the extent to which adaptation actually occurs will determine not only the success of establishment and control of the target species but also the extent of non-target effects. To predict potential non-target effects, researchers often use information concerning the phylogenetic relatedness of targets and non-targets (see Meyer *et al.*, 2008, this proceedings). Evolutionary processes in biological control are not limited to non-target effects. In fact, evolutionary processes can inform most procedures in classical biological control, from agent selection, to quarantine and pre-release studies, to establishment and other agent–target interactions (Table 1). Evolutionary predictions can also assist in cost–benefit and risk analyses. However, few biological control practitioners would consider conducting evolutionary studies to improve a biological control programme. Why is this the case?

There are a number of reasons why the importance of evolutionary processes in classical biological control has not been recognized. Evolution can be defined as a genetic change from one generation to the next. As such, many studies of evolutionary processes can take a long time or longer than can be accommodated within a funding cycle for a typical biological control programme. Fortunately, some processes, such as the intensity of natural or artificial selection or heritability, can be estimated within a generation. A second difficulty in understanding the role of evolution is the difficulty of conducting experimental tests of the phenomenon. For example, to assess whether an organism has

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**Table 1.** Evolutionary considerations in the procedures associated with classical biological control and the themes in this proceedings that relate to these procedures.

| Procedure | ISBCW Theme[a] | Evolutionary concepts | Types of studies |
|---|---|---|---|
| Agent selection | 5 | Phylogenetic hypotheses | Using relatedness in choice of agents and predicted host use |
| Quarantine | 6 | Population bottlenecks | Avoiding population bottlenecks through sampling design and out-crossing |
| Pre-release studies | 6 | Identification and diagnostics | DNA-based species identification (DNA barcoding); determining population origins; tracking individuals in experiments |
| Establishment | 1 | Genetic variation, effective population size, adaptation | Potentially maximizing genetic variation through maintaining large effective population sizes and out-crossing; understanding role of adaptation to new conditions |
| Post-release studies | 7 | Identification and diagnostics | DNA-based species identification (DNA barcoding); determining population origins; tracking individuals in experiments |
| Performance | 6,7 | Selection, adaptation | Evolutionary response to selection; estimating heritability for relevant traits, such as host use |
| Agent-target interactions | 6,7 | Selection, adaptation, co-evolution | Evolutionary response to selection; estimating heritability for relevant traits, such as host use |
| Non-target effects | 3,4,8 | Selection, adaptation | Estimating and predicting response selection, or lack thereof, for physiological tolerance or use of novel hosts |
| Risk analysis | 3,4,8 | Selection, adaptation | Predicting response to selection |

[a] ISBCW Themes: *1* ecology and modelling, *3* benefit–risk–cost analysis, *4* regulations and public awareness, *5* target and agent selection, *6* pre-release, specificity and efficacy testing, *7* release activities, *8* management specifics.

adapted to a novel host in a new habitat, ideally, one would like to conduct a reciprocal transplant involving both the source populations and introduced populations. With a few exceptions (e.g. Hufbauer, 2002), such experiments are typically not feasible. Nevertheless, useful information can be gained from less elaborate designs than reciprocal transplants, and much can be learned from so-called natural experiments. Finally, even when manipulations are possible, some field conditions are not easily replicated under laboratory or controlled settings. There is no easy solution to this problem. However, as discussed below, each classical biological control programme is an experiment in itself and follow-up studies or long-term monitoring can be used to analyze what works and what does not.

## Adaptation

Adaptation by biological control agents to new habitats not only can increase establishment and success but also can lead to undesired non-target effects (Hufbauer and Roderick, 2005). Adaptation is an evolutionary process caused by natural or artificial selection and is relatively simple to measure in the laboratory or field. One way to measure selection is by estimating the selection differential, $S$, which is the difference of the average phenotype of organisms after and before a selective event, such as the performance on a new host plant compared to an existing host. Estimates of selection differentials can be compared across studies and systems. To estimate whether a trait has a genetic basis, one can measure also the response to selection, $R$, which is the difference in the average phenotype in the generation after selection and the previous generation. The ratio of $R/S$ is the heritability, $h^2$, which is also an estimate of the proportion of phenotypic variation that has a genetic basis. There are other ways to measure heritability and other genetic parameters using slightly more elaborate experimental designs, such as by following related cohorts relative to their parents (parent–offspring regression) or by splitting families to estimate maternal effects (1/2-sib design).

Heritability can be used to measure and predict evolutionary responses in the field. For example, Cotter and Edwards (2006) raised 1/2-sib families of the moth, *Helicoverpa armigera* (Hübner), on resistant and susceptible chickpeas and found that host use was highly heritable, but the response also depended on the larval stage (instar) and host resistance. Even when breeding studies are not possible, one can test for evolutionary change. For example, Zangerl and Berenbaum (2005) used herbarium specimens over a span of 150 years to show that the phytochemistry of the invasive wild parsnip changed after introduction of the parsnip web-

worm. Indeed, many studies of herbivorous insects and plants have demonstrated evolutionary responses associated with colonization as a result of biological invasions (Müller-Scharer, 2006; Strauss *et al.*, 2006), and the same approaches can be used in studies of biological control. Likely, the most opportune time to conduct such studies is in the course of rearing for quarantine or pre-release testing. However, one can also estimate evolutionary responses over longer periods, such as associated with long-term monitoring.

## Genetic variability

Genetic variability is the raw material on which selection acts and so a reduction of genetic variability, such as in a population bottleneck, can result in a reduction in fitness as a result of inbreeding and a reduction in the ability of organisms to respond to new environmental conditions. In theory, issues related to population size and accompanying genetic variability are important in all aspects of biological control, from the initial collections and quarantine populations, to the individuals released and the potential for adaptation and non-target effects. Surprisingly, although introduced populations typically have lower genetic variability than their source populations (but see, Kolbe *et al.*, 2004; Marrs *et al.*, 2008), there is less evidence to show that genetic variation limits population growth in introduced populations (Roderick and Navajas, 2003; Hufbauer and Roderick, 2005). Several factors may explain this apparent conundrum. First, it may be that we have not observed the species or populations that were not successful as a result of low genetic variability, perhaps because they died out before observations were possible. Second, theoretical studies show that, although founding populations do lose alleles, particularly the rarer alleles, if populations can rebound quickly after introduction, the loss of overall genetic variability (measured as heterozygosity) can be minimized (Nei *et al.*, 1975). Finally, a series of explanations have been proposed to explain how small founding populations may recover genetic variation through genetic mechanisms, such as through conversion of epistatic variation (see Carson, 1990), greater effects of sex-linked genes (Whitlock and Wade, 1995), founder-flush phenomena where genetic drift is weaker in growing populations (Slatkin, 1996) and multiple introductions (Kolbe *et al.*, 2004). These effects have not yet been studied in biological control situations.

The effects of low population size may be both ecological and genetic. For example, experimental studies have shown that the probability of population establishment increases with release size (Grevstad, 1999). The Allee effect, which is a decline in population growth associated with low population size (Stephens and Sutherland, 1999), has been often evoked to explain this. However, recent experimental manipulation of initial densities of an invading parasitoid have shown that a number of life-history traits, including reproductive rate and sex ratio at low population densities, can protect populations from Allee effects (Fauverguee *et al.*, 2007).

## What can one predict?

Taken together, studies of adaptation and genetic variability in small, introduced populations can be used to make several predictions about changes that might be expected in biological control agents after introduction to a new environment.

1. Theory and empirical evidence from studies of biological invasions suggest that adaptation to old and new hosts and to new local environmental conditions should be common. However, few data are available to test this notion in biological control programmes.
2. Lack of genetic variation has been shown to have little impact on the success of introduced species and is not likely to be limiting in many biological control programs. This prediction is also very testable and very relevant to developing strategies for agent sampling, quarantine studies and release strategies.
3. Micro- and macro-organisms used for classical biological control may differ fundamentally in the extent to which adaptive change is important. For example, with their increased reproductive rate relative to their hosts, microorganisms adapt more quickly to novel hosts compared to macro-organisms. Studies to date of plant pathogens appear to support this notion (Roderick and Navajas, 2003).

# Methods and results

The methods of evolutionary biology include observations, manipulations, breeding studies and inferences based on genetic variability. Several texts in evolutionary biology and population genetics discuss these methodologies in detail (Table 2). In this paper, we focus on four advances that have been made in related fields, not necessarily directly connected to assessment of evolutionary change, but from which future studies of the role of evolution in biological control will clearly benefit.

## DNA barcoding

Many applications in biology, including biological control, require accurate and timely species identification (Navajas and Roderick, 2008). As species are generally thought to be interbreeding units separated from other such units, individuals within species will be more alike genetically than individuals of different species. This is the basis for an emerging tool called DNA barcoding, in which a small section of DNA can be used for species diagnostics (Savolainen *et al.*, 2005). For many taxa, this approach works extremely well, e.g. the

**Table 2.** An introduction to the literature in evolutionary biology and population genetics relevant to classical biological control.

| Topic | Information | References |
|---|---|---|
| Evolutionary biology textbook | College text in evolutionary biology | Futuyma, 2005 |
| Population genetics textbooks | Approachable population genetics texts geared toward applications | Falconer and MacKay, 1996; Hartl, 2000; Conner, 2004; Lowe *et al.*, 2004 |
| Phylogenetic methods | Advancing methods in phylogenetics, coalescence, and parameters estimated from these analyses | Emerson *et al.*, 2001; Rosenberg and Nordborg, 2002; Baldauf, 2003; Holder and Lewis, 2003; Hall, 2007 |
| Population genetic methods | Advancing methods in population biology and population genetics and parameters estimated from these methods | Beaumont and Rannala, 2004; Manel *et al.*, 2005; Excoffier and Heckel, 2006; Noor and Feder, 2006 |
| Molecular genetic markers and their uses | Description of molecular markers and their uses for evolutionary biology, population biology and diagnostics | Avise, 2004; Roderick, 2004; Schlötterer, 2004; Armstrong and Ball, 2005; Savolainen *et al.*, 2005; Navajas and Roderick, 2008 |
| Overviews | Reviews of evolutionary and population genetic concepts in biological control, biological invasions, and the use of historical collections | Roderick, 1992; Hopper *et al.*, 1993; Ehler, 1998; Fagan *et al.*, 2002; Roderick and Navajas, 2003; Suarez and Tsutsui, 2004; Hufbauer and Roderick, 2005; Strauss *et al.*, 2006; Sax *et al.*, 2007; Vellend *et al.*, 2007 |

use of mitochondrial cytochrome oxidase I in insects, although there are some complications, as for example with individuals of hybrid origin, species that have only recently diverged from one another and larger groups of taxa for which single genetic markers cannot reliably distinguish species, e.g. plants. Nevertheless, DNA barcoding can be an effective diagnostic tool for many needs in biological control, such as linking unknown larvae with adults, associating males and females of the same species, identifying parasitoid species in their hosts and making rapid taxonomic identifications. While genetic identification of species is not new, the novel focus on DNA barcoding worldwide is resulting in new collections and catalogues of species and extensive DNA databases with vouchered specimens deposited in museums, including species of interest to biological control. These specimens and their associated ecological and genetic data can provide baseline data for long-term studies that are critical to understanding the role of evolution in biological control.

## Whole genome sequencing

Advances in DNA sequencing technology and corresponding bioinformatics tools of DNA fragment assembly and annotation have made it possible to obtain nearly entire DNA genome sequences for a handful of so-called model organisms, including human, mouse, the puffer fish *Takifugu rubripes* Temminck and Schlegel, the nematode worm *Caenorhabditis elegans* Maupas, the wild mustard *Arabidopsis thaliana* (L.) Heynh., the fruit fly *Drosophila melanogaster* Meigen, the honeybee *Apis mellifera* Linnaeus and a growing number of other species. These genomes provide the opportunity to identify many genes of functional significance (Gomez-Zurita and Galian, 2005) and to develop a wealth of molecular markers for population genetic studies (Bouck and Vision, 2007) as well as to resolve evolutionary relationships between taxa (Savard *et al.*, 2006). Because biological control agents are typically not model organisms, biological control may not benefit as much from the genome boom as other biological disciplines. Nevertheless, we should expect the identification of homologous genes from model organisms that have relevance for biological control. For example, a DNA sequencing project of the first arthropod herbivore, the mite *Tetranychus urticae*, is nearly completed (Grbic *et al.*, 2007), and data from this project should lead to a better understanding of the genetics of host plant interactions. It is likely that the same loci can be examined in other herbivorous arthropods.

## Distributed databases

Recent progress has been made in databasing the information from specimen collection events, making it possible for anyone to use these data through a *distributed database* over the Internet. The core idea is that each collection or natural history museum curates and 'owns' its own specimens and is a *provider*, each deciding what to make publicly available. Then, if the different collections use similar fields in their data structure, a researcher can query data across all the participating providers simultaneously. The underlying software infrastructure supporting these tools is the collection management system already in use for

many museums worldwide (for a working example, see the Berkeley Natural History Museums, BNHM, biodiversity science initiative, http://bscit.berkeley.edu/). These systems are designed to be modular and flexible such that individual collections can easily link to and interact with other resources both within each museum and offsite, such as collections at other museums. Many styles of interaction can be supported, including machine-friendly methods, e.g. URL-based queries, formatted data dumps and DiGIR, Darwin Core, XML protocols and more human-friendly methods, such as linked interactive keys. Geospatial visualization, or mapping the data, can also be performed, e.g. using BerkeleyMapper and DiGIRMap. Many specimens also include a wealth of other information, including host use and habitat variables that can also be linked to such databases (Suarez and Tsutsui, 2004). For biological control, distributed databases can provide worldwide information on potential agents, including indigenous and introduced ranges. Coupled with climate or other ecological data, distributional data can be used to predict future spread and physiological tolerance. Target and non-target organisms can be investigated in a similar way.

### New computational tools

Computational tools for both population genetics and phylogenetics are advancing rapidly. In addition to bioinformatics through which enormous quantities of genetic data can now be processed and summarized (noted above), new approaches are emerging for both phylogenetic reconstruction (Baldauf, 2003; Noor and Feder, 2006) and population genetics (Emerson *et al.*, 2001; Manel *et al.*, 2005; Excoffier and Heckel, 2006). One promising avenue has been the use of Bayesian statistical methods to test a diversity of hypotheses in both areas (Holder and Lewis, 2003; Beaumont and Rannala, 2004). New phylogenetic approaches should improve biological control through more accurate assessments of species relationships for agent selection. Knowledge of host and habitat use of close relatives can be used to minimize non-target effects. Advances in population genetics are providing novel ways to trace the history of populations and to infer and predict demographic parameters useful for biological control programmes, such as population growth, geographical spread and habitat suitability.

## Discussion

One goal of this paper is to raise awareness of evolutionary biology and related fields so that new approaches can be used to better inform biological control programmes. A number of researchers have argued that recognizing general principles in the field of biological control can help move the discipline to more of a predictive science from one of a set of individual case studies (see discussions in Kareiva, 1996; Simberloff and Stiling, 1996; Holt and Hochberg, 1997; Hochberg and Gotelli, 2005). Certainly, evolutionary principles can help in this regard, as noted above for choosing agents and in better understanding the roles of adaptation and genetic variation. New methodologies provide additional data for study and new ways to examine existing data. Yet, many desirable manipulations are difficult, if even feasible. In this paper, biological control can likely benefit from the discipline of invasion biology, which also focuses on introductions and colonizations, establishment and spread (see Fagan *et al.*, 2002). Particularly important to both fields is a better understanding of successes and failures, which necessarily demands follow-up studies or long-term monitoring.

### Relevance of invasion biology

Classical biological control can be thought of as a series of semi-replicated field manipulations in which organisms are transplanted into new environments. It is well recognized that this process shares much with biological invasions (McEvoy *et al.*, 2008, this proceedings; Warner *et al.*, 2008, this proceedings) in that the introduced organisms typically find abundant resources and few predators, parasites or competitors (Ehler, 1998; Roderick and Navajas, 2003; Müller-Scharer, 2006). Both disciplines offer the possibility to follow long-term effects of introductions/colonizations. To make use of these studies, it is important to follow the introductions/colonizations from the beginning. In this regard, classical biological control has a distinct advantage in that introductions are well planned. In addition, to understand what factors determine which introductions will be successful, it is important to monitor not only well-known successes, but also failures. Unfortunately, after-the-fact studies are typically difficult to fund, although one might argue that they are the most important (McFadyen, 2008, this proceedings). Certainly, long-term evolutionary responses associated with classical biological control or invasion biology can only be understood when historical data or specimens are available for comparison.

### Suggestions for further study

To understand better evolutionary processes in classical biological control, we can make several recommendations that are relevant to agent selection, quarantine, pre- and post-release studies, establishment, agents–target interactions, non-target effects and risk analysis. The following are the suggestions for further study:

1. Involve researchers from several disciplines in biological control efforts. This review has focussed on

evolutionary biology where the recommendation is particularly relevant, but biological control programmes would also benefit from those studying risk and social science.

2. Preserve and database pre-release samples and associated ecological data. Pre-release data collections with vouchers are critical both for future ecological and genetic studies. We recommend that the specimens be accessioned to a well-maintained national or regional collection or natural history museum, which has a mission of long-term storage. For some questions, specimens also can provide information on habitat variables, including competitors, predators and pathogens (Suarez and Tsutsui, 2004). This is a requirement of some countries, e.g. Australia, when introducing a potential biological control agent for quarantine studies.
3. Conduct genetic studies in the context of rearing in quarantine and pre-release testing. If rearing studies are being conducted and if it is possible to have replicates within the rearing design, by keeping track of parentage, it is possible to conduct simple studies of heritability for host use and for other traits. Such data is invaluable for predicting host use and could be obtained with little extra work.
4. Emphasize long-term monitoring. Genetic markers have been proven to be useful to monitor the fate of released individuals (Navajas *et al.*, 2001). Long-term monitoring of classical biological efforts will benefit many sub-disciplines, not just evolutionary biology. Risk assessment is one area in which real-world data are lacking, e.g. McFadyen (2008, this proceedings). Such monitoring is not easy, is often difficult to fund and will likely take a coordinated effort of researchers.
5. Publish on all biological control introductions, including those that do not work for whatever reason. By publishing results of unsuccessful introductions, we can compare what does work with what does not and hence assess variables underlying success. Admittedly, publishing on unsuccessful introductions will take some change in editorial policies in respected journals. Towards the same goal, researchers can present research on unsuccessful introductions in a broader hypothetical framework, such as invasion biology, so that negative results can be more interesting and relevant.

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# Does phylogeny explain the host-choice behaviour of potential biological control agents for Brassicaceae weeds?

**H.L. Hinz[1], M. Schwarzländer[2] and J. Gaskin[3]**

*Summary*

Four invasive Brassicaceae are currently being studied at CABI Europe-Switzerland for biological control. A phylogenetic approach to host testing has so far been hampered by the fact that the evolutionary relationships of taxa within the Brassicaceae were unclear. Recently, a new phylogeny of the Brassicaceae, largely based on molecular studies, has been proposed. This presents a unique opportunity to relate host-range test results for some of our Brassicaceae agents to the new phylogeny. The host range of *Ceutorhynchus scrobicollis* Nerensheimer & Wagner, a root-crown mining weevil investigated as a potential agent for garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande, appeared to closely follow the new classification (significant linear relationship between phylogenetic distance and host-range test results). However, for *Ceutorhynchus cardariae* Korotyaev, a gall-inducing weevil considered as biocontrol agent for hoary cress, *Lepidium draba* L., phylogenetic distance of the test species to the target weed did not explain a significant amount of the variation in host preference or suitability. These results question the general applicability of the centrifugal phylogenetic method, where it is assumed that species more closely related to the target are at greater risk of attack than species more distantly related. The importance of other factors, specifically secondary metabolite profiles and morphological characteristics for the host-choice behaviour of *C. cardariae* are currently being investigated.

***Keywords***: *Alliaria petiolata*, *Lepidium draba*, centrifugal phylogenetic method.

## Introduction

To date, no biological control agents have been released against weeds in the mustard family (Brassicaceae). The main reason for this is the family's large number of economically important crop species and its many genera indigenous to North America. Four invasive Brassicaceae are currently being studied at CABI Europe-Switzerland for biological control. They are garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande, hoary cress, *Lepidium draba* L., perennial pepperweed, *Lepidium latifolium* L. (Hinz *et al.*, 2008, this proceedings), and dyer's woad, *Isatis tinctoria* L. (Cortat *et al.*, 2008, this proceedings).

In biological control of weeds, it is generally assumed that species closely related to the target are at greater risk of attack than species more distantly related. However, a phylogenetic approach to host testing has so far been hampered by the fact that the evolutionary relationships of taxa within the Brassicaceae were unclear. The subdivision of the Brassicaceae at the tribal and subtribal levels has been a controversial aspect in the systematics of the family (Appel and Al-Shehbaz, 2003). Appel and Al-Shehbaz (2003) concluded that 'in the absence of comprehensive, family-wide molecular data it is not regarded advisable to propose or recommend any classification system'. Recently, Al-Shehbaz *et al.* (2006) and Bailey *et al.* (2006) proposed the long-awaited new tribal alignment of the Brassicaceae based on molecular studies and careful evaluation of morphology and generic circumscriptions. This presented a unique opportunity to see whether host-range test results for some of our Brassicaceae agents correlated with the new phylogeny of the Brassicaceae. We used host-specificity test results of two currently studied potential biocontrol agents, viz., *Ceutorhynchus scro-*

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*bicollis* Nerensheimer & Wagner (Coleoptera, Curculionidae) on *A. petiolata* and *Ceutorhynchus cardariae* Korotyaev on *L. draba,* and correlated them with the genetic distance of test plant species to the respective target weed.

# Methods and materials

## The study species

*A. petiolata* is a strict biennial European herb introduced into North America that, by 2000, had spread to 34 states and four Canadian provinces (Blossey *et al.*, 2001). *A. petiolata* is one of the few introduced herbaceous species that invades and dominates the understory of forested areas in North America. In 1998, a biological control program was started, and four weevils were prioritized as potential agents. One of these is the root-crown mining weevil, *C. scrobicollis*. Adults of this species aestivate in summer. Oviposition on *A. petiolata* rosettes begins in mid-September and lasts until the beginning of April of the following year (Gerber *et al.*, 2007). Eggs are laid into petioles and leaves, and the growing point and larvae mine through petioles towards the roots and develop mainly in root crowns, occasionally also in shoot bases. Mature larvae leave host plants in spring to pupate in the soil, and the next generation of adults emerges in May and June.

*L. draba* [=*Cardaria draba* (L.) Desv.], with its two subspecies, *L. draba* spp. *draba* and *L. draba* spp. *chalapense*, and its close relative *Lepidium appelianum* Al-Shehbaz [=*Cardaria pubescens* (C.A. Mey.) Jarm.], are perennial mustards (Brassicaceae) of European origin that were introduced in the USA in the late 19th century (Lyons, 1998). Since then, they spread throughout the western and the northeastern states and are now declared noxious weeds in 16 states and three Canadian provinces (Rice, 2005). Because they are difficult to control sustainably using mechanical or chemical methods, a consortium was established in Spring 2001 to investigate the scope for classical biological control. Five insect species are currently being studied for the biological control of *L. draba*: four weevils and one flea beetle. One of these is the gall-inducing weevil *C. cardariae* Korotyaev. Females of *C. cardariae* lay their eggs in the leaf midribs, petioles and developing shoots of *L. draba* from early spring until mid-June (Hinz *et al.*, 2007). Oviposition induces the formation of galls, in which the larvae mine and develop. Mature larvae leave the plants to pupate in the soil, and the next generation of adults emerges from May to July.

## Host-specificity tests

***A. petiolata–C. scrobicollis*:** Between 1999 and 2006, sequential no-choice oviposition tests were conducted with *C. scrobicollis*. A mated pair of *C. scrobicollis* was placed into a transparent plastic cylinder (11 cm diameter, 15 cm high) and alternately offered cut leaves of *A. petiolata,* then those of a test-plant species. After 3 to 4 days, the plant material was removed, checked for feeding marks, dissected for eggs and the weevils provided with fresh plant material. Each exposure period was treated as one replicate. A replicate for test plants was only regarded as valid when the female laid at least one egg into the test plant or into an *A. petiolata* plant following a test plant.

Plant species accepted for oviposition were subsequently exposed in no-choice oviposition and development tests. Two to three females and one to two males were released onto individually potted, gauze-covered rosettes of *A. petiolata* or onto test species. To verify that females were fertile, one pair was offered a cut leaf of garlic mustard in a cylinder for 2 to 3 days after which plant material was dissected for eggs. Only females that laid eggs on these leaves were used for the tests. For each no-choice experiment, two to 13 garlic mustard plants were infested concurrently as controls. After 2 to 4 weeks, weevils were retrieved and plants re-covered with gauze bags. In late spring of the following year, all plants were searched regularly for emerging adults until emergence ceased.

***L. draba–C. cardariae*:** Between 2003 and 2006, no-choice oviposition and development tests were conducted with *C. cardariae*. One to five females and one to four males, depending on plant size, were placed onto individually potted, gauze-covered test plants or *L. draba* (control plants). All females were tested for egg-laying before use in tests (see above). Each time a series of test plants was infested, two to four *L. draba* plants were infested concurrently as controls. After 8 to 12 days, weevils were retrieved from the plants, and feeding, oviposition and gall formation were recorded. To ensure that females had a chance to feed and oviposit on *L. draba* in between tests, all weevils were placed into cylinders for a couple of days and provided with cut plant material of *L. draba* before beginning a new series of tests. About 1 month after infestation, plants were rechecked for gall development. After about 12 weeks, all plants were checked for adult emergence.

## Molecular and statistical analyses

Leaf material of plant species within the family Brassicaceae used in tests with *C. scrobicollis* and *C. cardariae* that were not included in the phylogenetic tree of Beilstein *et al.* (2006) was collected and subjected to molecular analysis. Genomic DNA was isolated using a cetyl trimethylammonium bromide method. Polymerase chain reaction (PCR) amplification of the chloroplast *ndhF* region was done with same primers and conditions as in Beilstein *et al.* (2006). PCR products were purified using QIAquick PCR Purification kit (Qiagen) before sequencing in a Beckman CEQ 2000XL automated sequencer using standard protocols including the LFR-1 method of injection time and volt-

age. Sequences were aligned manually using Se–Al (Rambaut, 1996). Maximum parsimony (MP) analysis was performed and uncorrected (*p*) distances of the data set were determined using PAUP* v. 4.0b8 (Swofford, 2000). For estimation of the most parsimonious phylogenetic trees, the heuristic MP search employed 500 random taxon addition sequences and the tree-bisection-reconnection branch-swapping algorithm. All characters were weighted equally. A 10,000-replicate, fast stepwise-addition, bootstrap analysis was conducted to assess clade support. The phylogenetic analysis was included to illustrate evolutionary relationships of the plant taxa, while the distance measurements were correlated with host-specificity measurements.

To relate results of host-specificity tests (for *C. scrobicollis*, the number of eggs laid and the number of offspring produced per female; for *C. cardariae*, the number of galls induced and offspring produced per female) with the genetic distances generated for test plants of each target weed species, we used simple linear regression analysis. The two target weeds were not included in the analyses, as the aim was to test whether plants more closely related to the respective target weed would be preferred by female weevils for oviposition and/or would be more suitable for weevil development. When data on the genetic distance for a test-plant species was not available, we used congeners of known genetic distance from the target to extrapolate the missing value to the precision of two decimal places.

# Results

## *A. petiolata–C. scrobicollis*

Of the 28 plant species and varieties for which data on both oviposition-test results and genetic distance were available, 18 were accepted for oviposition by *C. scrobicollis* females (Fig. 1A). As expected, females of *C. scrobicollis* laid more eggs on plants more closely related to *A. petiolata* ($r^2$ = 0.298, $F_{1,27}$ = 11.02,

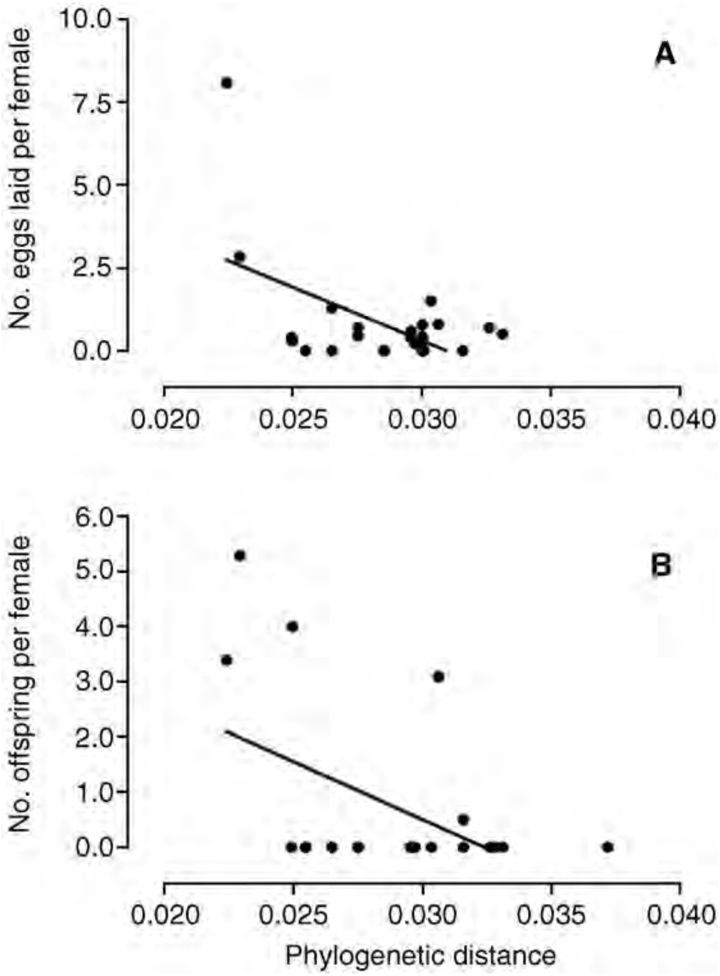


**Figure 1.** Relationship between genetic distance of test-plant species to the control (i.e. target weed) *Alliaria petiolata* and (A) the number of eggs laid and (B) the number of offspring produced per *Ceutorhynchus scrobicollis* female on the respective test-plant species.

$P = 0.003$) than on less closely related plants. The two plant species that received the most eggs, the European species, *Peltaria alliacea* Jacq. and *Thlaspi arvense* L., are in the same tribe as *A. petiolata* (Thlaspideae), according to the new molecular phylogeny (Al-Shehbaz *et al.*, 2006; Fig. 2). No plant species outside the family Brassicaceae supported normal oviposition behaviour of *C. scrobicollis* (Gerber *et al.*, 2005).

Of the 21 plant species and varieties for which data on both results of no-choice development tests and genetic distance were available, adults emerged from five species other than *A. petiolata*, viz. the European species, *Nasturtium officinale* R.Br., *P. alliacea*, and *T. arvense*, and the North American species, *Rorippa sinuata* (Nutt.) A.S. Hitchc. In addition, a single adult in a single replicate emerged from the commercially grown cabbage variety *Brassica oleracea sabauda* 'Paradisler'. However, none of the other six *B. oleracea* varieties we offered were attacked (Gerber *et al.*, 2005), and moreover, no attack occurred under multiple-choice cage conditions. This suggests that *B. oleracea* and its various cultivars are outside the fundamental

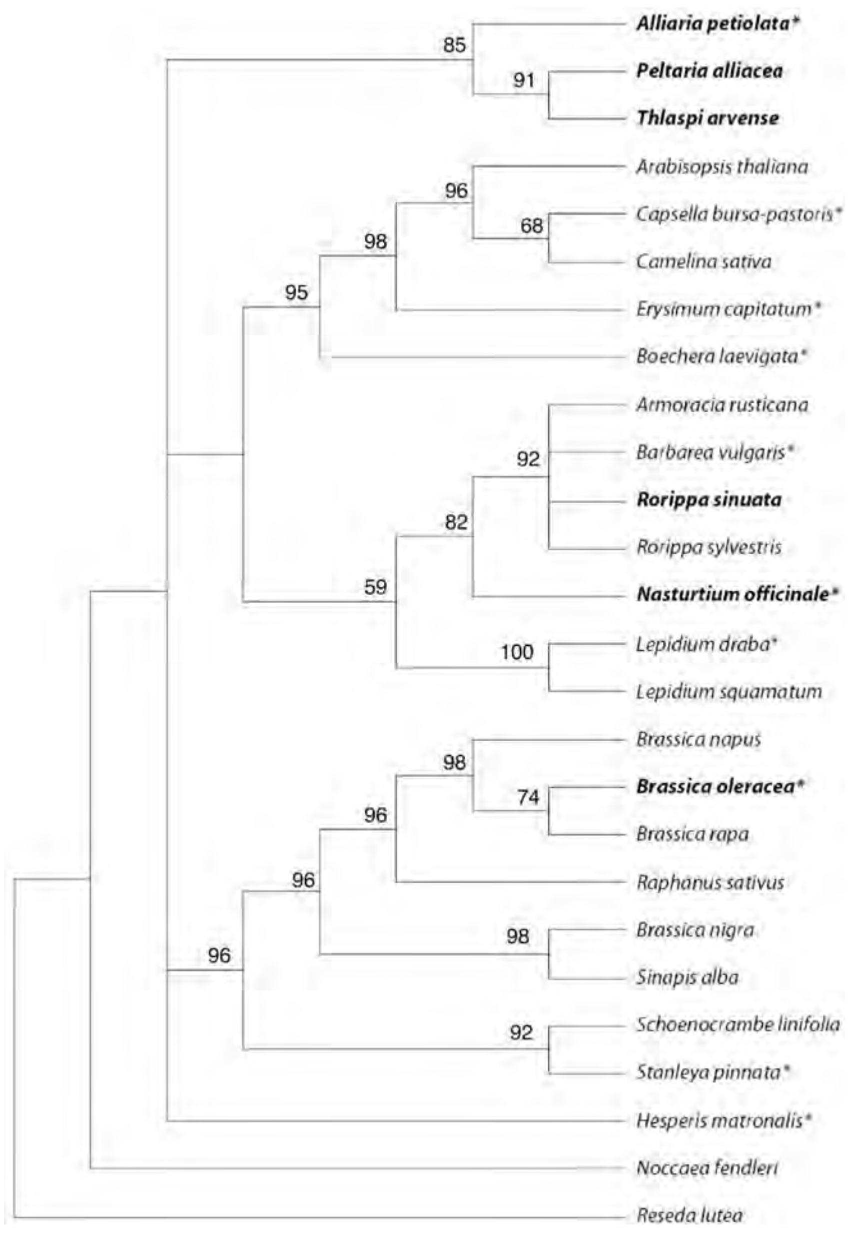


**Figure 2.** Phylogeny of *Alliaria petiolata* and test-plant species used in oviposition and development tests with *Ceutorhynchus scrobicollis*. The figure is a strict consensus of the ten most parsimonious trees, each 615 steps in length, derived from 1965 aligned bases of the chloroplast gene *ndhF*. Bootstrap values (>50%) are shown *above branches*. Taxa that supported adult development are shown in *bold font*. An *asterisk* indicates that the DNA sequence was provided by M. Beilstein.

host range of *C. scrobicollis*. We therefore consider the emergence of a single adult an artefact of experimental conditions. Females of *C. scrobicollis* produced more offspring on plants more closely related to *A. petiolata* ($r^2 = 0.237$, $F_{1,20} = 5.91$, $P = 0.025$; Figs. 1B and 2) than on less closely related species.

It is worth noting that the North American species, *Noccaea fendleri* (A. Gray) Holub (see Fig. 2), was formerly known as *Thlaspi montanum* L. (Thlaspidae), until all North American species of *Thlaspi* were recently assigned to the tribe Noccaeeae, genus *Noccaea*, to form a monophyletic group separate from the European species of *Thlaspi* (Koch and Al-Shehbaz, 2004; also see Fig. 2). Host-specificity results with *C. scrobicollis* supported this new classification.

## *L. draba–C. cardariae*

Of the 58 plant species for which data on both host-specificity test results and genetic distance were available, galls were induced on 11 species and adults emerged from ten (Fig. 3A, B), including the second target weed *L. appelianum*. In contrast to *C. scrobicollis* on *A. petiolata*, neither the number of galls induced nor the number of offspring produced per *C. cardariae* female was correlated with genetic distance of test-plant species from the target weed *L. draba* (number of galls induced, $r^2 = 0.025$, $F_{1,57} = 1.46$, $P = 0.232$; number of offspring produced, $r^2 = 0.020$, $F_{1,57} = 1.15$, $P = 0.287$). Plant species that supported gall induction and the development of adults included the fairly distantly related species *Caulanthus anceps* Payson and *C. inflatus* S. Wats. (the genetic distance for both of which was extrapolated from *C. crassicaulis*, see 'Methods and materials' for details), *Stanleya pinnata* (Pursh) Britt. and *Lobularia maritima* (L.) Desv. (Fig. 4). While only one adult each emerged from each of the latter two species, a similar number of adults emerged from *C. anceps* and *C. inflatus* as from *L. draba* control plants (Hinz *et al.*, 2007). In contrast, *S. viridiflora* Nutt. did not support gall induction and was minimally fed on by *C. cardariae*.

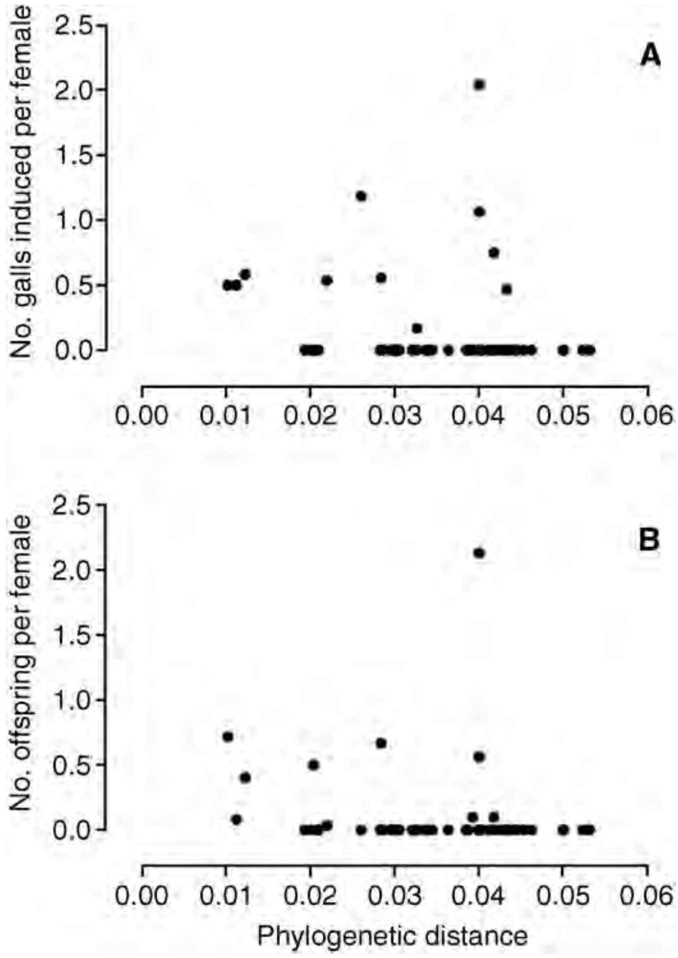


**Figure 3.** Relationship between genetic distance of test-plant species to the control (i.e. target weed) *Lepidium draba* and (A) the number of galls induced and (B) the number of offspring produced per *Ceutorhynchus cardariae* female on the respective test-plant species.

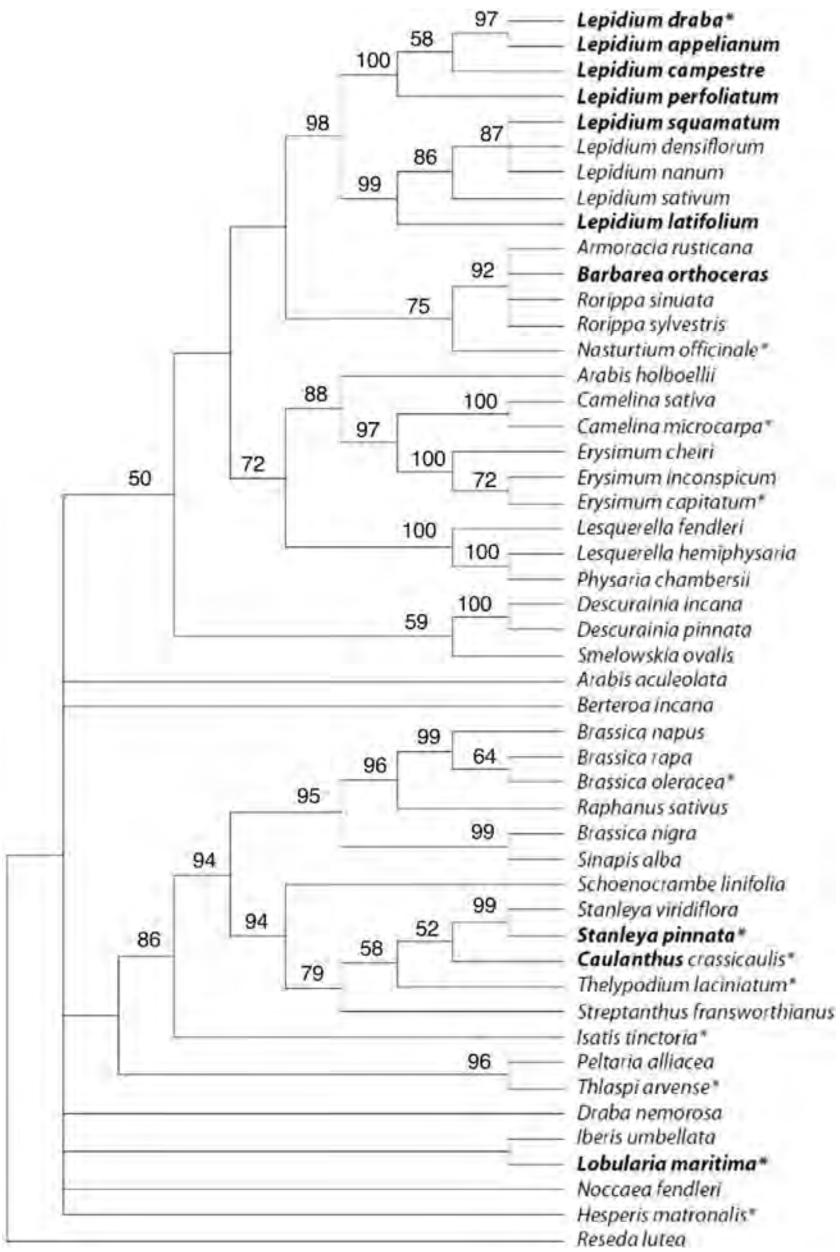


**Figure 4.** Phylogeny of *Lepidium draba* and test-plant species (used in oviposition and development tests with *Ceutorhynchus cardariae*). The figure is a strict consensus of the 54 most parsimonious trees, each 999 steps in length, derived from 2017 aligned bases of the chloroplast gene *ndhF*. Bootstrap values (>50%) are shown *above branches*. Taxa that supported adult development are shown in bold font. An *asterisk* indicates that the DNA sequence was provided by H. Beilstein.

## Discussion

In three multiple-choice, field-cage tests established between 2004 and 2006 with *C. cardariae*, in which several test species were exposed that had supported development under no-choice conditions, only the three target weeds, i.e. the two subspecies *L. draba* spp. *draba* and *L. draba* spp. *chalapense* and *L. appelianum* were attacked, indicating a very narrow host range for the weevil under multiple-choice conditions. However, plants that supported development under no-choice conditions constitute the physiological host range of a species, which in turn appears to be an effective criterion for identifying species potentially at risk of attack, as there is no example of an insect agent attacking a plant outside its physiological host range after release (Pemberton, 2000; van Klinken and Edwards, 2002).

In North America alone, the family Brassicaceae is represented by approximately 600 species in more than 35 endemic genera. Also in North America, there

are at least 123 Brassicaceae species within 23 genera that are considered economically important. Our host-specificity test results showed that none of the most widespread and important economic Brassicaceae species are at risk of attack by either biological control candidate. In addition, if commercially grown Brassicaceae would be part of the normal host range of any of our agents tested, the species would have been recorded as a pest in the European literature, which is not the case (Schwarz *et al.*, 1990). However, native North American Brassicaceae have never been previously exposed to the insects studied here, and the large number of plant species makes it difficult, if not impossible, to test them all. We were therefore hoping to use the new Brassicaceae phylogeny to select plant species for additional host-specificity tests and to extrapolate the risk of non-target attack on more distantly related genera to the respective target weeds.

The host range of *C. scrobicollis*, investigated for the biological control of *A. petiolata*, only appears to include species closely related to the target. In contrast, *C. cardariae* on *L. draba* appears to have a disjunct host range with some distantly related plants supporting development to a similar degree as the control under no-choice conditions. These results are at odds with the centrifugal phylogenetic method (Wapshere, 1974), where it is generally assumed that species closely related to the target are at greater risk of attack than species more distantly related. In the absence of detailed, explicit phylogenies, the centrifugal phylogenetic method has usually been based on traditional taxonomic classifications, which has been questioned (Briese and Walker, 2002; Kelch and McClay, 2003). However, our study was based on the latest molecular phylogeny of the Brassicaceae.

For *Longitarsus jacobaeae* Waterhouse, which was studied for the biological control of *Senecio jacobaea* L., neither adult feeding nor the fundamental larval host range of *L. jacobaeae* were clearly predicted by the phylogeny of the genus *Senecio* (U. Schaffner, unpublished data). In contrast, it was found that leaf dry matter content of *Senecio* species explained a significant amount of the variability in the amount of leaf area eaten by adult beetles (U. Schaffner, unpublished data). Another study found that the functional composition of herbivore assemblages on 18 shrubs was correlated with respective leaf structural traits (Peeters, 2002). More specifically, leaf trichome density and leaf surface waxes have been shown to influence host suitability or preference of insect herbivores (Eigenbrode and Espelie, 1995; Levin, 1973, and Edwards, 1982, in Peeters, 2002). Finally, secondary plant compounds play an important role in host finding, host acceptance and host suitability of insect herbivores (e.g. Renwick, 1989; Rask *et al.*, 2000). We are currently investigating some of these potential factors to better explain the host acceptance and suitability patterns observed for *C. cardariae*. Because host acceptance and suitability of herbivores might be influenced by a combination of many different secondary compounds and physical characteristics (see above), we will be using a relatively new technique, i.e. metabolomics that will allow us to identify and quantify all metabolites (primary and secondary) of an organism simultaneously (Bezemer and van Dam, 2005 and refs therein).

In conclusion, it is not our intention to question the importance of phylogenetic relatedness to understanding host-choice behaviour of herbivorous insects. Indeed, since the general adoption of the centrifugal phylogenetic method for host-range testing of weed biological control candidates (Wapshere, 1974), there has not been a single significant case of an agent that attacked a non-target that was completely unanticipated or unpredicted (Pemberton, 2000). We do, however, propose that other factors influence the host choice behaviour of insect herbviores more than commonly considered. As already suggested previously (e.g. Keller, 1999; Withers; 1999; Briese, 2005), these factors need to be better understood and should be included in the selection of test plant species and in the interpretation of host-specificity test results.

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# Population structure of an inadvertently introduced biological control agent of toadflaxes: *Brachypterolus pulicarius* in North America

**R.A. Hufbauer[1] and D.K. MacKinnon[1,2]**

*Summary*

Phytophagous insect species that use more than one host plant often harbour distinct populations specialized on different host species. *Brachypterolus pulicarius* L. (Kateridae) is considered to be a biological control agent of Dalmatian and yellow toadflax, *Linaria dalmatica* (L.) P. Mill. and *Linaria vulgaris* P. Mill., in North America. We evaluated the population structure of this beetle using microsatellite loci to determine whether beetles collected from the two hosts differed. We found no significant microsatellite variation attributable to host plant. These results corroborate previous ecological results showing that beetles collected from both hosts have similar preferences: They prefer yellow toadflax and generally perform better on it as well.

***Keywords***: *Linaria*, plant–insect interactions, microsatellite.

## Introduction

Many phytophagous insect species that feed on more than one host are comprised of genetically differentiated populations that are specialized on individual host plants or even genotypes of host plants (Fox and Morrow, 1981; Mopper and Strauss, 1998). In biological control, such specialization has the potential to increase efficacy and perhaps safety when there is a good match between the target weed, which is the host plant, and the biological control agent (Goolsby *et al.*, 2004, 2006). Alternatively, specialization may inhibit successful control if there is not a good match (Lym *et al.*, 1996; Lym and Carlson, 2002) or if the plant has the upper hand in the evolutionary arms race with its enemies so that it has defences specific to genotypes of enemies attacking it (Kaltz *et al.*, 1999).

*Brachypterolus pulicarius* L. (Kateridae) is a flower-feeding beetle that attacks Dalmatian and yellow toadflax, *Linaria dalmatica* (L.) P. Mill., spp. *dalmatica* and *Linaria vulgaris* P. Mill., (Scrophulariaceae). It was inadvertently introduced into North America along with its host plants. These two toadflax species were initially brought to North America from Eurasia for their ornamental and medicinal properties but have since become invasive weeds. *B. pulicarius*, now considered to be a biological control agent, is able to reduce the seed set of both hosts dramatically under controlled conditions (McClay, 1992; Grubb *et al.*, 2002). It is common at high densities on yellow toadflax and is thought to contribute to successful biological control of that weed (MacKinnon *et al.*, 2005). It is not always present on Dalmatian toadflax, however, and when it is present, it is often found only at low densities (MacKinnon *et al.*, 2005, 2007). MacKinnon *et al.* (2005, 2007) have investigated the preference and performance of *B. pulicarius* on both hosts to determine whether this species is comprised of populations specialized on each host. They found that beetles collected from both hosts generally prefer yellow toadflax and also perform better on yellow toadflax. In this paper, we follow up on previous work by evaluating whether

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the molecular population structure of *B. pulicarius* in North America provides evidence for genetic differentiation on the two hosts.

## Methods and materials

We collected *B. pulicarius* from six infestations of Dalmatian toadflax and eight of yellow toadflax in western North America (Table 1). Additionally, we obtained samples from two European sites, one of each host plant (Table 1). Beetles were stored in 95% ethanol in a –20 or –80°C freezer before DNA extraction. We extracted genomic DNA using DNeasy™ tissue kits (Qiagen). To develop microsatellite markers, genomic DNA was digested with *Apo*I and *Bst*YI. The genomic library was constructed by ligating size-selected fragments (700–1600 bp) into pUC 19 that had been digested with *Bam*HI and *Eco*RI and gel-purified. Vectors were transformed into competent *Escherichia coli* cells (Life Technologies Max Efficiency DH5α) and plated on Luria–Bertani/ampicillin agar. Colonies were lifted with Magna Graph membranes (Osmonics, Inc.) and screened with a $^{33}$P-labeled probe of pooled oligonucleotides (AC and AG dimers and all possible trimers excluding homopolymers). Positive colonies were polymerase chain reaction (PCR) amplified with M13 forward or reverse primers and sequenced with a BigDye Terminator Cycle Sequencing Kit and an ABI 377 automated sequencer (PE Applied Biosystems). Primers were designed for 30 loci (GenBank accession nos. EU078572–EU078591), only four of which produced reliable and relatively easy to score amplification products (Table 2).

Microsatellite loci were amplified using PCR Express thermocyclers (Hybaid) in 10 µl reactions containing 1 µl genomic DNA, 1× PCR buffer (20 mM Tris–HCL, pH 8.4, 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM each deoxyribonucleotide triphosphate, 2 pmol of each primer, 0.5 units *Taq* polymerase (Life Technologies) and 0.1 µl TaqStart antibody (Clonetech). Amplification cycle conditions consisted of approximately 1 min at 90°C and then 35 cycles of 50 s at 95°C, 1 min at annealing temperature (Table 2), 1.5 min at 72°C and then a final extension step for 45 min at 72°C. Reactions were held at 0–4°C before separation in an ABI 3100 capillary instrument.

We ran basic statistics to evaluate Hardy–Weinberg equilibrium and linkage disequilibrium on the microsatellite data using GenePop on the Web (http://genepop.curtin.edu.au/; Raymond and Rousset, 1995). We looked for evidence that a bottleneck in population size has reduced the number of rare alleles using both the stepwise and infinite allele mutation models in the program Bottleneck (Cornuet and Luikart, 1996; Piry *et al.*,1999). We evaluated population structure between regions (North America and Europe) and within North America due to sampling location and host plant, with analyses of molecular variance (AMOVA) in Arlequin version 3.01 (Excoffier *et al.*, 2005). We evaluated whether there is a relationship between geographic and genetic distance (isolation by distance) by implementing a Mantel test in IBDWS (http://www.bio.sdsu.

**Table 1.** Collection locations, host plant, approximate GPS coordinates, and sample sizes (*n*) for *Brachypterolus pulicarius*.

| Plant species location | Coordinates[a] | *n* |
|---|---|---|
| ***Dalmatian toadflax*** | | |
| Christina Lake, BC, Canada | 49.03′00″N, 118.12′00″W | 22 |
| Kamloops, BC, Canada | 50.42′00″N, 120.23′00″W | 23 |
| Cheyenne, WY | 41.06′12″N, 104.53′22″W | 28 |
| Gillette, WY | 44.17′34″N, 105.30′19″W | 23 |
| Steamboat Springs, CO | 40.29′27″N, 106.49′03″W | 45 |
| Necedeh, WI | 44.01′00″N, 90.04′00″W | 24 |
| Macedonia[b] | | 11 |
| ***Yellow toadflax*** | | |
| Donald, BC, Canada | 51.29′00″N, 117.09′00″W | 26 |
| Edmonton, AB, Canada | 53.29′02″N, 113.29′51″W | 17 |
| Rosalind, AB, Canada | 52.47′00″N, 112.26′00″W | 19 |
| Afton, WY | 42.45′01″N, 110.57′17″W | 11 |
| Poudre Park, CO | 40.42′00″N, 105.16′00″W | 12 |
| Estes Park, CO | 40.23′06″N, 105.32′33″W | 37 |
| Steamboat Springs, CO | 40.29′57″N, 106.49′15″W | 17 |
| Buckhorn Park, WI | 43.56′00″N, 89.59′00″W | 21 |
| Rhine Valley, Germany[2] | | 9 |

[a] Coordinates without minutes (indicated as 00) are approximate.
[b] European samples were provided without precise locality data.

**Table 2.** Characteristics of four microsatellite loci isolated from *Brachypterolus pulicarius* including locus name (clone number) and GenBank accession number, primer sequences, PCR annealing temperature ($T_a$), repeat motif in cloned allele, the size of the sequenced allele, the number of alleles found, the size range of the amplified alleles, observed heterozygosity ($H_O$) and expected heterozygosity ($H_E$). The top primer was dye-labeled for visualization.

| Locus (accession) | Primer sequences (5′–3′) | $T_a$ (°C) | Repeat of cloned allele | Size (bp) | Number of alleles | Size range (bp) | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|
| 5 | AACTGAC-CAGCGTTAAAT-GATAAT | 60 | $(GA)_{13}$ | 304 | 5 | 317–325 | 0.15 | 0.31 |
| (EU078586) | AGAGTGAATATT-GTCCCTTCTCAA | | | | | | | |
| 16 | ATTAT-CAGCTCCACA-GAAAACACC | 55 | $(AG)_{15}$ | 263 | 8 | 235–263 | 0.26 | 0.31 |
| (EU078591) | ATATAAGT-TCACGTTCG-GGGTTTG | | | | | | | |
| 19 | TGAG-GCCAACTAAACT-TCAGA | 55 | $(GT)_8$ | 139 | 11 | 125–157 | 0.20 | 0.37 |
| (EU078590) | GACTCGAG-GGCAGATA-CAATC | | | | | | | |
| 37 | ACTGCCAAAC-CAAGTC-CAAAACT | 65 | $(CT)_{22}$ | 331 | 15 | 335–379 | 0.08 | 0.35 |
| (EU078589) | GTTGGTTGCTT-TCTCGGC | | | | | | | |

edu/pub/andy/IBD.html; Bohonak, 2002; Jensen *et al.*, 2005).

## Results and discussion

None of the four loci were in Hardy–Weinberg equilibrium: All showed a significant deficit of heterozygotes ($P < 0.0001$ across all loci and populations, Table 2). This indicates that at least one evolutionary process (selection, drift, migration and non-random mating) is influencing the populations sampled. While this is not in itself surprising, particularly for an introduced species, it is notable that the pattern is consistent across locations, given that it takes only a single generation for Hardy–Weinberg equilibrium to be established. In addition, two of the loci (16 and 19) were in linkage disequilibrium ($P < 0.0001$), suggesting physical linkage, selection or non-random mating.

Despite the deficit of heterozygotes, the program Bottleneck generally revealed an L-shaped distribution of alleles for all loci and population combinations. This suggests that a bottleneck in population size associated with the introduction of the beetles into North America did not last long enough to lead to the loss of substantial numbers of rare alleles or that multiple introductions occurred. Bottleneck should ideally be used with 20 or more loci, however; thus, the lack of evidence for a bottleneck should be considered a preliminary finding. With only four loci, the power of those tests was low.

The lack of Hardy–Weinberg equilibrium breaks the basic assumptions of AMOVA. While AMOVA is fairly robust to such issues, the following analyses should be interpreted cautiously. The AMOVA comparing the native and introduced range showed significant differentiation between North American and European sample locations (Table 3). This differentiation suggests that the inadvertent introduction of *B. pulicarius* into North America was from somewhere other than the collection sites we tested from the Rhine Valley and Macedonia. Alternatively, differences in the selective regime or other evolutionary processes could have led to differentiation of North American and European samples.

The AMOVA examining the variation within North American samples showed that most of the variation was within populations, as is typical for microsatellite loci (Table 3). There were also significant differences among collection locations, revealing significant population structuring. Despite this, the Mantel test found no relationship between geographic and genetic distance across North America ($r = 0.13$, $P = 0.18$), suggesting either high mobility or insufficient time for a balance between drift and migration to establish, both of which are likely in this system.

Finally, AMOVA provided no evidence that beetles from the two host plants were genetically differentiated

**Table 3.** Analyses of molecular variance (AMOVA).

| Source of variation | *df* | Sums of squares | Variance components | Percent of variation |
|---|---|---|---|---|
| Partitioning of variation across European and North American samples, by region and location within region | | | | |
| Among regions | 1 | 10.64 | 0.122 | 16.90* |
| Among locations within regions | 13 | 15.86 | 0.014 | 1.96* |
| Within locations | 641 | 375.39 | 0.586 | 81.14** |
| Partitioning of variation within North America due to host plant at the collection location and among collection locations | | | | |
| Among host plants | 1 | 1.27 | –0.004 | –0.45 |
| Among locations within hosts | 12 | 28.06 | 0.033 | 3.43** |
| Within locations | 614 | 564.41 | 0.919 | 97.02** |

* $P$<0.01, ** $P$<0.001.

(Table 3). This finding fits well with previous results from this system, which showed that beetles from both hosts tend to prefer and perform better on yellow toadflax than Dalmatian toadflax (MacKinnon *et al.*, 2005, 2007). Thus, it appears that *B. pulicarius* is not comprised of distinct groups specialized on alternate hosts (Fox and Morrow, 1981) but, rather, is a yellow toadflax specialist, and use of Dalmatian toadflax is merely incidental. This likely will prevent *B. pulicarius* from contributing in a meaningful way to the control of Dalmatian toadflax.

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# Genetic analysis of native and introduced populations of *Taeniatherum caput-medusae* (Poaceae): implications for biological control

**S.J. Novak[1,2] and R. Sforza[3]**

*Summary*

Genetic analysis of both native and introduced populations of invasive species can be used to examine population origins and spread. Accurate delineation of an invasive species' source populations can contribute to the search for specific and effective biological control agents. Medusahead, *Taeniatherum caput-medusae* (L.) Nevski, a primarily self-pollinating Eurasian annual grass that was introduced in the western USA in the late 1800s, is now widely distributed in California, Idaho, Nevada, Oregon, Utah and Washington. The goal of our current research is to assess introduction dynamics and range expansion of this grass in the western USA, and to identify source populations in the native range to facilitate the search for potential biocontrol agents. Across introduced populations, nine multilocus genotypes were detected, and we suggest a minimum of seven separate introduction events of *T. caput-medusae* in the western USA. Although range expansion appears to have occurred primarily on a local level, several introduced populations appear to be composed of admixtures of introduced genotypes. None of the native populations analysed to date possess the exact multilocus genotypes detected in introduced populations. We have recently begun screening Eurasian populations using intersimple sequence repeat (ISSR) genetic markers to determine whether this polymerase chain reaction–based technique can provide a higher degree of resolution for the identification of source populations.

***Keywords***: invasive grass, multilocus genotypes, multiple introductions.

## Introduction

Experimental analyses of both native and introduced populations of invasive species can be used to assess various ecological, genetic and evolutionary aspects of invasion and the invasion process (Hierro *et al.*, 2005; Novak, 2007). For instance, comparison of the level and structure of genetic diversity within and among native and introduced populations can be used to determine whether the distribution of a species in its new range stems from single or multiple introduction events (Novak and Mack, 2001, 2005; Lavergne and Molofsky, 2007). Additionally, genetic analysis of native and introduced populations can be used to examine population origins and spread (Roderick and Navajas, 2003). Accurate delineation of an invasive species' source populations (or regions) can contribute to the search for biological control agents. Indeed, the identification of areas of origin may reduce the economic cost of prospecting for agents and may result in the development of more specific and effective biological control agents (Goolsby *et al.*, 2006a).

*Taeniatherum caput-medusae* (L.) Nevski, a member of the tribe Triticeae in the grass family, is considered a noxious weed in many western US states (e.g. Colorado, California, Oregon, Nevada and Utah). The grass was first collected in the USA in Roseburg, Oregon in 1887 (Fig. 1), and its collection history is well documented (McKell *et al.*, 1962; Young, 1992). The grass has now invaded millions of hectares of semi-arid

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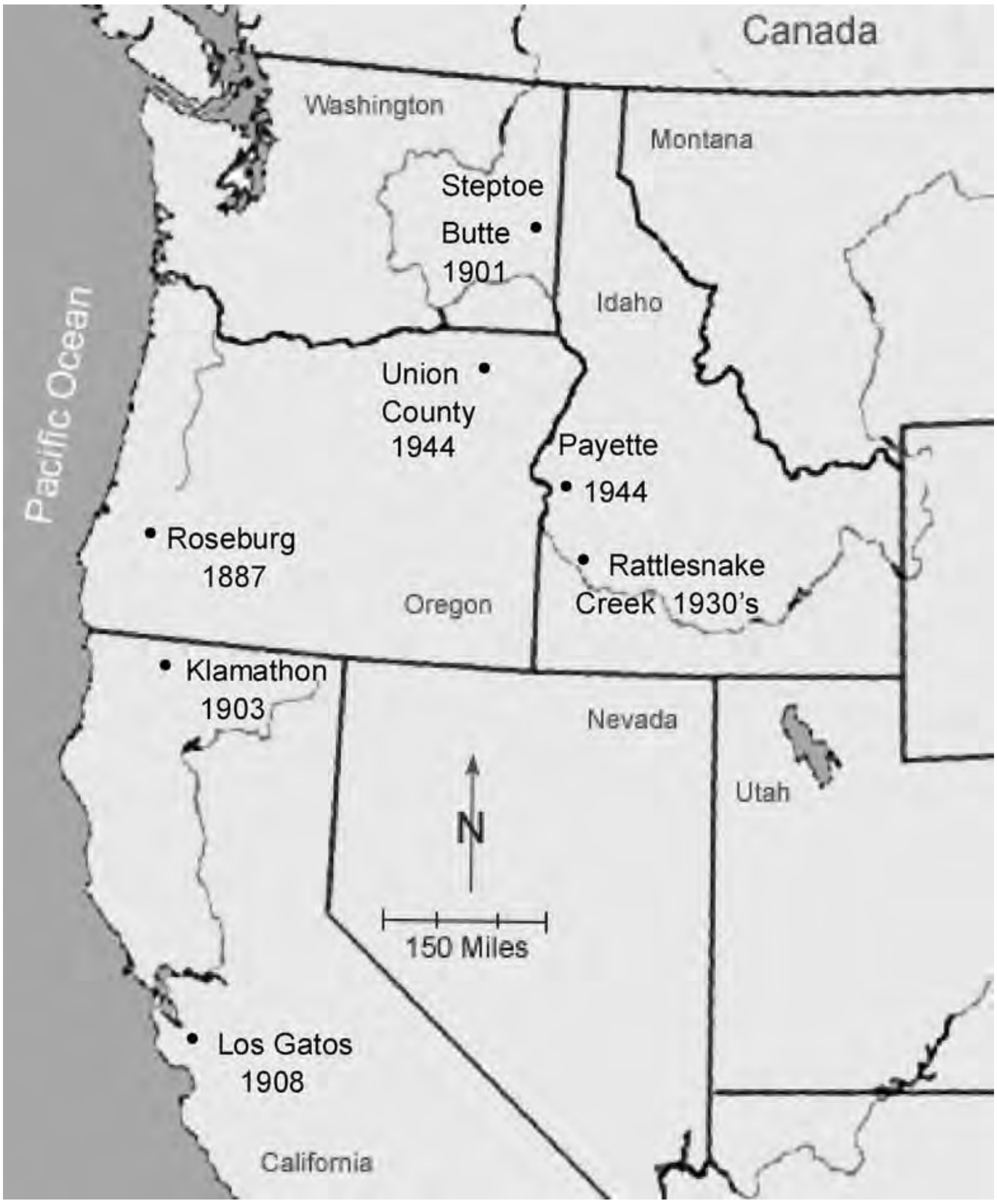


**Figure 1.** Chronology of the spread of *Taeniatherum caput-medusae* in the western USA. This chronology was reconstructed using published accounts (see text), herbarium specimens and historical

rangeland in the western USA (Young, 1992; Miller *et al.*, 1999). It primary occurs in areas disturbed by overgrazing and fire in the 25–100 cm annual precipitation zone, and it can become the dominant plant species at certain sites (Hironaka, 1961; Dahl and Tisdale, 1975; Young, 1992). Ominously, the species has probably not yet reached its ecological limit. If its ecological requirements approximate those of *Bromus tectorum* L., it has the potential to spread widely in the Great Basin of the USA and beyond. Different methods have been tried to control *T. caput-medusae* (burning, grazing, competition and herbicides), and all have generally resulted in failure (Horton, 1991).

The native range of *T. caput-medusae* includes much of Eurasia, where three distinct subspecies have been recognized (Frederiksen, 1986), but only *T. caput-medusae* ssp. *asperum* is believed to have been introduced into the USA (Young, 1992). Recently, foreign exploration was carried out for identifying candidates for biological control, and several plant pathogens were described, including the fungi, *Ustilago phrygica* Magnus and *Tilletia bornmuelleri* Magnus (Siegwart *et al.*, 2003; Widmer and Sforza, 2004). A preliminary host range screening with *U. phrygica*, a systematic smut fungi that was collected in Turkey and attacks *T. caput-medusae*, has been conducted (Sforza *et al.*, 2004).

Because natural enemy pressure can vary across genotypes (Evans and Gomez, 2003), populations and regions, the enemy release hypothesis is best tested by comparing introduced populations with native populations

that were the source of the invasion. When implementing biological control programs to reverse the release from natural enemies, knowledge of source populations or regions would increase the probability of finding highly specialized enemies (Goolsby *et al.*, 2006b). In addition, evaluating the efficacy of candidate biological control agents should be done on the full range of genotypic diversity present within the introduced range (Gaskin *et al.*, 2005), through the evaluation of specific polymerase chain reaction (PCR) primers when screening populations (Marrs *et al.*, 2006).

The overall goals of our current research are to assess introduction dynamics and range expansion of *T. caput-medusae* in the western USA and to identify source populations in the species' native range to facilitate the search for potential biocontrol agents. This research specifically addresses the following questions. (1) How many multilocus genotypes occur in western US populations of *T. caput-medusae*, and what is their geographic distribution? (2) What does the distribution of these genotypes indicate about range expansion of this species in its new territory? (3) How many genotypes occur in native range populations, and what is their geographic distribution? (4) Can source populations for the invasion of the grass in the western USA be identified?

# Methods and materials

## Sampling of plant material

One objective of our sampling has been to collect plant material from populations across the entire geographic distribution of *T. caput-medusae*, in both its invasive and native ranges. Another objective has been to obtain population samples at or near localities where the plant was first collected, or reported, during its invasion of the western USA (Fig. 1). Samples have been collected from a total of 45 populations in the states of California, Idaho, Nevada, Oregon, Utah and Washington, with several early collection localities represented.

Two groups of native range samples have been included in this study. The first group consisted of 23 populations, with 22 of these populations being accessions obtained from the USDA Plant Introduction Laboratory in Pullman, WA: 12 populations from Turkey, seven from Afghanistan, two from Iran and one from Kazakhstan. The personnel of the USDA Plant Introduction Laboratory variously classified these accessions to each of the three subspecies of *T. caput-medusae*. Only one of these 23 populations originated in Europe: Sterea Hellas, Greece. The second group consisted of 49 populations collected in August or September, 2002 and 2003, from across the grasses' native range in Eurasia. For most populations, intact spikes were collected from 30 to 40 individual plants along a transect at approximately 1-m intervals and placed in separate envelopes. Seeds from the 49 Eurasian populations were brought back to the United States Department of Agriculture Agricultural Research Service European Biological Control Laboratory (USDA-ARS-EBCL) in Montpellier, France, on an official authorization (04LR011) granted by the French government. Seeds were stored in a quarantine greenhouse at the EBCL until further use.

## Enzyme electrophoresis

The level and structure of genetic diversity within and among populations of *T. caput-medusae* in its invasive range in the western USA is based on the analysis of 1663 individuals from 45 populations. In the laboratory, one seed from each individual in a population was germinated on moistened filter paper in a Petri dish and harvested approximately 7 days after germination. Enzyme electrophoresis was conducted generally following the methods of Soltis *et al.* (1983), with modifications described by Novak *et al.* (1991). The 15 enzymes employed in this study were resolved with enzyme electrophoresis using four buffer systems (1, 6, 8 and 9), and these 15 enzymes were genetically encoded by 29 loci. Because *T. caput-medusae* is a diploid with low genetic diversity, the genetic basis of all allozyme variation observed was easily inferred based on known subunit structure and compartmentalization of these enzymes (Weeden and Wendel, 1989).

## ISSR analysis

Five of the 49 Eurasian populations of *T. caput-medusae* mentioned above were selected for a preliminary analysis using intersimple sequence repeat (ISSR) genetic markers: one population from Morocco, Spain, France, Greece (Crete) and Turkey. For each population, five seeds were randomly selected from each of three plants located 5, 18 and 25 m along the transect from which they were sampled. In the EBCL quarantine greenhouse, seeds were germinated in Petri dishes with distilled water at 25°C, 80% relative humidity and 16:8 h light/dark. Ten days after germination, leaves were removed from the plants and frozen at –20°C. Total genomic DNA was extracted from frozen leaf material using DNeasy Plant Mini Kits according to the manufacturer's instructions (Qiagen Inc., Valencia, CA). After extraction, DNA was amplified with the PCR using six ISSR primers decribed by Wolfe *et al.* (1998). Primer names and sequences are provided in Table 1. DNA amplifications were performed in 20 µl final reaction volumes containing 1 U of Taq DNA polymerase (Qiagen Inc.), 1× buffer (Qiagen), 1 mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphates, 0.5 µM of a single primer and 2 µl of the template DNA. Amplifications were performed using the GeneAmp PCR System 9700 (Applied Biosystems, Forest City, CA) as follows: 94°C for 3 min, then 35 cycles at 94°C for 30s, 45°C for 45 s and and 72°C

**Table 1.** Identity and nucleotide sequences of the ISSR primers used in this preliminary analysis of *Taeniatherum caput-medusae* from its native range. ISSR primers used in this study were described by Wolfe *et al.* (1998). The utility of each primer, based on the criteria described in the text, is indicated: *Y* yes, *N* no.

| Primer | Primer sequence | Utility |
|---|---|---|
| ISSR-17898A | $(CA)_7$–AC | Y |
| ISSR-17898B | $(CA)_7$–GT | N |
| ISSR-17899A | $(CA)_7$–AG | Y |
| ISSR-17899B | $(CA)_7$–GG | Y |
| ISSR-814 | $(CT)_8$–TG | Y |
| ISSR-HB15 | GTG–GTG–GTG–GC | N |

for 1 1/2 min. A final extension was performed at 72°C for 7 min. Amplified products were electrophoresed on 1.0% agarose gels. Gels were stained with ethidium bromide, and DNA fragments were visualized with a UV transilluminator and photographed.

## Data analysis

Allozyme multilocus genotypes were identified from enzyme electrophoresis data, and these genotypes were used to assess introduction dynamics and spread of *T. caput-medusae* in the western USA and to identify source populations of the grass in its native range. Allozyme multilocus genotypes are defined as the composite genotype over all loci examined and therefore are designated based on the identity of alleles at each scored enzyme locus. Populations were defined as genetically polymorphic if they contained two or more multilocus genotypes. As part of our preliminary analysis of native populations of *T. caput-medusae* using ISSR genetic markers, bands were not scored; however, we did qualitatively assess each primer to determine its utility for future analysis. Specifically, primers were evaluated based on whether they (1) did not generate bands in control reactions, (2) generated clear, distinct, darkly stained bands and (3) were polymorphic among test populations.

# Results

## Multilocus genotypes in the introduced range

Multilocus genotypes are named based on the populations in which they were first found. A total of nine multilocus genotypes were detected among all 45 populations: seven homozygous multilocus genotypes and two genotypes with one or two heterozygous loci (unpublished data, not shown). The seven homozygous genotypes were first detected in Roseburg, OR, Steptoe Butte, WA, Rattlesnake Station, ID, Ladd Canyon, OR, Pullman, WA, Malloy Prairie, WA and Salt Creek, UT. Five different multilocus genotypes were observed in the Palouse region of eastern Washington. The multilocus genotypes detected in Pullman, Malloy Prairie and Salt Creek appear to be restricted to just a single population. Heterozygous multilocus genotypes were found in two different populations: White Bird, ID contained two heterozygous genotypes and one of these genotypes was also detected at Emigrant Hill, OR. The level of polymorphisms within introduced populations was low: Only 17 of 45 populations (37.8%) contained two or more multilocus genotypes. Furthermore, of these 17 polymorphic populations, only three contained three or more multilocus genotypes.

## Multilocus genotypes in the native range

Two distinct categories of multilocus genotypes were detected within the 23 Eurasian populations. The first group of genotypes were quite distinct and differed from those detected in the western USA at multiple loci. These genotypes were present in seven populations from Turkey, and all populations from Afghanistan, Iran and Kazakhstan. Based on their different enzyme banding patterns, plus their larger seed size, these populations probably all consisted of *T. caput-medusae* ssp. *crinitum*. The enzyme multilocus genotypes detected in the remaining populations in Turkey and the one from Greece were similar to those observed in the western USA, and these populations were tentatively assigned to *T. caput-medusae* ssp. *asperum*. However, none of the multilocus genotypes present in these 23 native populations was an exact match to those previously detected in the introduced range.

## Preliminary ISSR analysis

Of the six ISSR primers that were screened in our preliminary analysis of native populations of *T. caput-medusae*, four met our selection criteria and will prove useful in future studies of both native and introduced populations (Table 1). Samples from the five countries (populations) display different DNA banding patterns with primer ISSR-17899A (Fig. 2), although no variability was detected among individuals within populations.

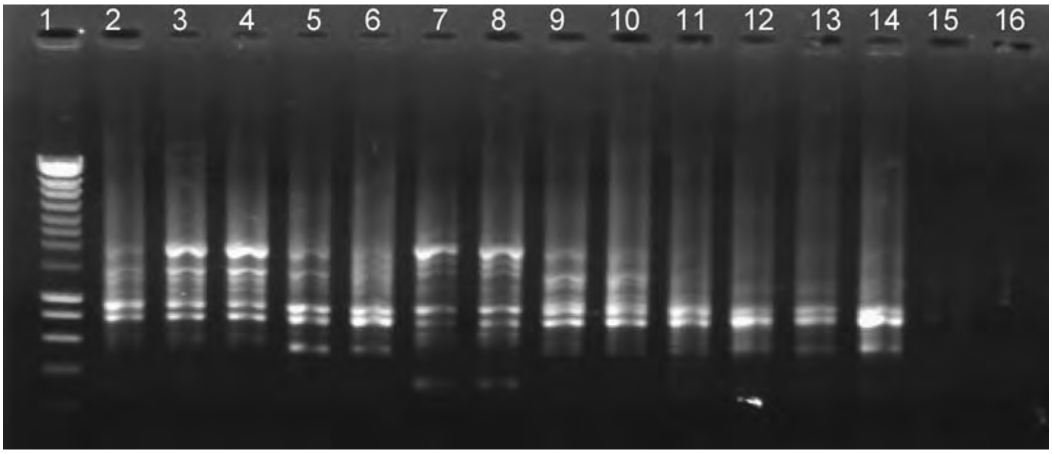


**Figure 2.** Photograph of DNA banding patterns obtained using ISSR primer 17899A. Note the different banding patterns for populations of *Taeniatherum caput-medusae* from different countries. Contents of the lanes on this gel are as follows: *1* 1 kb ladder; *2–4* France; *5* and *6*, Greece; *7* and *8*, Morocco; *9–11*, Spain; *12–14*, Turkey; *15* PCR control; *16* extraction control.

# Discussion

## Introduction dynamics and spread in the western USA

The level of genetic diversity observed across and within western US populations of *T. caput-medusae* is lower than the mean value reported for other self-pollinating plant species (Hamrick and Godt, 1990) but similar to that of other invasive plants that exhibit a uniparental mode of reproduction such as selfing (Novak *et al.*, 1991). Yet, despite its lack of genetic diversity, at least at the loci examined in this study, this species is now invasive over much of the semi-arid portions of the western USA.

The occurrence and geographic distribution of multilocus genotypes can provide insights into introduction dynamics and spread of invasive species (Novak and Mack, 2001, 2005). Multilocus genotype results for western US populations of *T. caput-medusae* are consistent with the pattern often associated with multiple introductions. Based on just the number of homozygous multilocus genotypes detected across all populations, we suggest a minimum of seven independent founder events. This conclusion is bolstered by the observation that four of the localities where these genotypes were detected are at or near early collection sites of the plant: Roseburg (1887), Steptoe Butte (1901), Rattlesnake Station (1930) and Ladd Canyon (1944).

The detection of five different multilocus genotypes in eastern Washington suggests that multiple introductions can occur within a relatively small geographic area. Because the plant was not collected in Utah until 1988, the detection of a unique multilocus genotype in Salt Creek, Utah may be evidence for a relatively recent introduction event. If so, these data indicate that introduction of this grass is ongoing. Our results for *T. caput-medusae* join a growing body of information indicating that, for invasive plant species, multiple introduction may be the rule rather than the exception (Novak and Mack, 2005) and may contribute to invasiveness (Allendorf and Lundquist, 2003; Lavergne and Molofsky, 2007; Novak, 2007).

The low level of polymorphisms observed within introduced populations of *T. caput-medusae* indicates that gene flow or dispersal among these populations is low. Thus, we conclude that spread or range expansion of the species has occurred mostly at the local or regional level and certainly has not been widespread. However, the detection of populations that appear to be admixtures of different introduced genotypes, as seen at several locations in the western USA (data not shown), suggests that intermixing of genotypes can take place if multiple introductions have occurred within the same region. Moreover, the detection of heterozygous multilocus genotypes suggests that plants with different genotypes and potentially originating from different introduction events have recently mated. Such hybridization events have been suggested to contribute to increased invasiveness in introduced species (Ellstrand and Schierenbeck, 2000).

## Source populations

Although the multilocus genotyes observed in populations from Greece and several from Turkey are similar to those of the western USA, no exact matches were found among native populations. Thus, our allozyme analysis did not reveal the source populations (or regions) for the introduction of *T. caput-medusae* in the USA, but the data clearly excludes many of the southwest and central Asian locations from serving as

source populations. These results probably stem from insufficient sampling in the native range, especially Europe: Only one of 23 native populations was from Europe, and the other 22 populations were collected from southwest or central Asia.

Additional analysis of European populations will be required before source populations, and introduction dynamics of the invasion of *T. caput-medusae* in the western USA can be more confidently described. To this end, our future plans include allozyme analysis of the 49 additional Eurasian populations, which have already been collected. In addition, our preliminary analysis of native range populations of the grass using ISSR genetic markers is very promising and will hopefully provide us with PCR-based markers that possesses high degrees of polymorphism and resolution for identifying source populations.

### Prospects for biological control

Foreign exploration for the identification of possible biological control agents has already led to the identification of several promising plant pathogens (Siegwart *et al.*, 2003; Widmer and Sforza, 2004). The genetic analyses described in this paper are meant to compliment this effort, and results of these analyses reveal the likelihood for biological control of *T. caput-medusae*. Introduced populations of the grass are genetically depauperate; thus, we would anticipate fast population build-up and spread of highly adapted biocontrol agents (Müller-Schärer *et al.*, 2004). However, multiple introductions, the occurrence of some introduced populations that are genetic admixtures and the detection of low level of outcrossing within a few populations means that several biological control agents from different portions of the native range may be required (Burdon and Marshall, 1981). Thus, the accurate identification of source populations is needed to augment the exploration for biological control agents already taking place and may result in the development of more specific and effective biological control agents for this highly destructive invasive species.

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# The use of surrogate herbivores for the pre-release efficacy screening of biological control agents of *Lepidium draba*

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*Summary*

Pre-release efficacy assessment has been suggested as a primary selection criterion for potential biological control species to insure the success and safety of biological weed control. Pre-release efficacy of candidate agents is commonly assessed by exposing insects to target plants in the garden or greenhouse (native range) or in quarantine (introduced range) before or in parallel with host-specificity testing. Conducting pre-release impact experiments for several candidate agents simultaneously may be difficult because potential agents are either scarce or may require development of novel culturing procedures. We propose an alternative approach to pre-release efficacy assessment that utilizes oligophagous or polyphagous insect herbivores from the introduced range as surrogates for biological control agents to assess the impact of specific feeding niches on the target weed to direct the search for effective candidate agents. Surrogate herbivores can be cosmopolitan or indigenous insects collected directly from the invasive plant and confamilial species. Insect pest species are particularly well suited to act as surrogates because they are seasonally abundant or easily reared. Based on previous surveys, we identified surrogate herbivores attacking different above-ground plant organs of the biological control target hoary cress (*Lepidium draba* L., Brassicaceae). We tested the density-dependent impact of four oligophagous herbivore species on *L. draba* to demonstrate the applicability of this novel efficacy assessment technique. We found that the endophagous stem miner, *Ceutorhynchus americanus* Buchanan (Coleoptera: Curculionidae), had the highest per-capita effect on hoary cress growth, suggesting candidate agents within this niche should be prioritized.

***Keywords***: herbivore niche, agent selection, generalist insects.

## Introduction

The biological control of weeds has been a successful, economical and environmentally sound management tool for curbing plant invasions, but McFadyen (2003) estimated that only 55% of biological control agents established contribute to the suppression and control of their target weeds. In addition, more than half of the successful biological control programs can link their success to a single agent (Denoth *et al.*, 2002). Introduction of ineffective biological control agents increases the probability of direct risks to non-target plant species, interference with established agents and indirect effects on ecosystem functions without the benefits gained from reduction of weed dominance or abundance. To minimize the cost of host-specificity screening and risks associated with establishment and proliferation of ineffective agents, improved methods for predicting the impact of candidate agent species before their release are recommended (Sheppard, 2003; McClay and Balciunas, 2005). Pre-release efficacy testing of candidates provides quantitative data to improve the likelihood of selecting the agents most capable of reducing weed abundance.

The most commonly used technique for pre-release efficacy assessment is to expose candidate agents to target plants in garden or greenhouse experiments in their native range or under quarantine conditions in their introduced range. The advantage of these methods is that changes in plant performance can be directly attributed to agents at experimentally controlled densities. However, screening several candidate species

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simultaneously may be particularly difficult because potential agents are often scarce initially or may require development of novel rearing protocols. Results of field experiments conducted in the area of origin may also be confounded by the use of weed genotypes different from those in the invaded range, interference by other herbivores unlikely to be utilized in biological control and competitive interactions with plant species that are not present in the invaded range.

Another technique that has recently been advocated is the use of simulated herbivory for pre-screening insect efficacy (Raghu and Dhileepan, 2005; Schooler *et al.*, 2006). The advantage of simulated herbivory is the ease of applying precise experimental treatments (Hjältén, 2004) without the need for insect quarantine. Although this approach has been shown to effectively mimic insect feeding impact in some cases, plant responses to mechanical damage do not always match the magnitude and quality of responses to herbivore damage (Lehtilä and Boalt, 2004). In addition, it can be difficult to artificially simulate the diversity of insect damage because of the unique spatial and temporal distribution of damage caused by each feeding mode.

The use of surrogate herbivores provides an alternative approach to pre-release efficacy testing that combines the strengths of actual insect herbivory and the convenience of simulated herbivory. Surrogate herbivores can be used early in the development of a biological control program for testing the sensitivity of the target plant to damage inflicted by different feeding niches, which could then guide the search for new insect agents. The protocols for subsequent pre-release efficacy testing of candidate agents can be established and the results compared with generalist herbivores of the same niche. The main advantage of this approach is that it can be conducted with resources abundant in the invaded range of the weed without the expense of quarantine facilities. Surrogate herbivores can be indigenous or cosmopolitan insects that occur in the introduced range of the weed. These insects are often seasonally abundant and easily collected from the habitats invaded by the target weed or from agricultural habitats. They may already utilize the target plant or be generalist pests of confamilial plant species. Several agricultural pest species have well-established mass-rearing protocols for laboratory colonies or are available for purchase.

We used surrogate herbivores to assess the potential effects of candidate biological control agents on hoary cress, *Lepidium draba* L. (Brassicaceae). An extensive survey of *L. draba* insects by Cripps *et al.* (2006) indicated that several above-ground feeding guilds are represented in North America by oligophagous native and polyphagous pest species. To test the impact of these species and to determine the most effective feeding guild for biological control, we investigated the response of hoary cress to four different types of oligophagous herbivore damage at different densities.

# Materials and methods

*L. draba* ssp. *draba* (=*Cardaria draba* (L.) Desv.) is a Eurasian perennial mustard introduced to North America in the 19th century (Mulligan and Findlay, 1974). *L. draba* is currently considered a noxious weed in 15 western states and three Canadian provinces (Rice, 2007). *L. draba* occurs in a wide range of habitats, including cultivated land, rangeland, wilderness, pastures, roadsides and waste areas, but thrives particularly well in disturbed, riparian or irrigated areas (Mulligan and Findlay, 1974). It reproduces sexually and vegetatively through rhizomes. Seeds usually germinate in early autumn and produce overwintering rosettes, which bolt in spring and flower from April to June in the northwestern USA.

Surrogate insect herbivores were selected based on the feeding modes of two specialist insects currently under consideration for biological control of *L. draba*: the stem-mining weevil, *Ceutorhynchus merkli* Korotyaev (Coleoptera: Curculionidae), and the stem and root-crown feeding flea beetle, *Psylliodes wrasei* Leonardi & Arnold (Coleoptera: Chrysomelidae) (Cripps *et al.*, 2006). The native stem-miner, *Ceutorhynchus americanus* Buchanan, was the only insect found mining in the shoots of *L. draba* in North America and was used as a surrogate for *C. merkli*. Adult *C. americanus* feed on foliage and oviposit in the stems of *L. draba* in laboratory no-choice tests (K.P. Puliafico, unpublished data). The host affinity of this native weevil is unknown, but adults have been collected from *Brassica* and *Lepidium* species and it has only been successfully reared from *Lepidium virginicum* (Buchanan, 1937).

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), was selected as a surrogate to mimic adult feeding of *P. wrasei*. Crucifer flea beetles are introduced Brassicaceae pests, which cause crop damage primarily from feeding on seedling plants but usually have only a minor effect on established plants (Feeny *et al.*, 1970).

Two additional insects were utilized as surrogates to mimic feeding modes for which there are currently no biological control candidate species: a lepidopteron defoliator and a piercing/sucking true bug. The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered native to the Mediterranean region but has a cosmopolitan distribution and occurs wherever crucifer crops are grown (Talekar and Shelton, 1993). This defoliating moth is oligophagous within the Brassicaceae (Talekar and Shelton, 1993) and is abundant on *L. draba* in North America (Cripps *et al.*, 2006). The tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), is native to North America and was one of the most abundant polyphagous species found on invasive *L. draba* (Cripps *et al.*, 2006). *Lygus* species lacerate plant tissue, inject salivary fluids that digest the tissue extra-orally and then ingest the liquefied tissue (Butts and Lamb, 1990).

This study was conducted at the climate-controlled Manis Entomological Greenhouse, University of Idaho, Moscow, ID. Environmental conditions were maintained with a 15L/9D photoperiod at 24 ± 2°C (day)/18 ± 1°C (night) in the greenhouse throughout all phases of the experiment except for the seedling vernalization described below.

*L. draba* seeds were collected from a population near Moscow, ID (46°44′N, 116°58′W) in August 2003. Seeds were sown into Cornell 'Peat-Lite A' artificial potting medium (Hartmann *et al.*, 1990) in the greenhouse. After formation of the first true leaves, seedlings were transplanted into plug trays using the same potting medium. Seedlings were grown for 6 weeks in the greenhouse and were then transferred to a 3 ± 1°C cold room with 12L/12D photoperiod for a minimum of 60 days to initiate bolting and flower production. During vernalization seedlings were fertilized once a week with 0.33 ml of Miracle-Gro® per litre of water (15-30-15 NPK, Scotts Miracle-Gro, Marysville, OH). After cold treatment, plants were transplanted into 3-l plastic pots in artificial potting media (1:2:1 peat moss, vermiculite and perlite mix; augmented with 5% sand, pH stabilizers and trace elements) and fertilized with 2.8 g Osmocote® slow release fertilizer per litre soil (14:14:14 NPK, Scotts Miracle-Gro, Marysville, OH). Plants were then grown in the greenhouse for 6 to 8 weeks before they were used for experimentation.

*C. americanus* was collected from *L. draba* near Vale, Oregon (44°05′N, 117°18′W) on 24 April 2004. Females were tested individually for oviposition on cut *L. draba* stems before the start of the experiment (Harmon and McCaffrey, 1997). *P. cruciferae* adults were collected on 29 April 2004 from *Sinapis alba* seedlings southeast of Genesee, ID (46°33′N, 116°55′W). Adult and nymph *L. hesperus* were collected from a mixed field of alfalfa and pasture grasses at the University of Idaho Sheep Research Farm, Moscow, ID (46°44′N, 116°58′W) 2 days before the start of the experiment on 12 August 2004. *P. xylostella* eggs were obtained from Benzon Research Inc. (Carlisle, PA). A laboratory colony was reared on artificial diet in 473-ml Styrofoam cups with plastic lids following the protocols of Shelton *et al.* (1991). Approximately 300 adults of both sexes were caged together, and females were allowed to oviposit for 24 to 48 h on cabbage-juice-coated aluminum foil. Foil was then cut into 1 $cm^2$ pieces; eggs were counted and combined into treatment densities, which then were placed at the base of the plants.

Stem number and individual stem lengths were measured for all plants before the start of experiments. Plants were assigned to herbivore treatments in a complete randomized block design with position on greenhouse bench as the blocking factor. Plants were caged with 80-cm-long mesh sleeve cages supported with internal wire frames with the bottom of each sleeve held in place with a metal hose clamp. Herbivores were released according to treatments (see below). All plants were caged for 40 to 44 days and then destructively harvested in blocks. Plants were clipped at the soil surface, the numbers of shoots counted and individual shoot lengths recorded to the nearest 1 cm. Roots were carefully washed to remove soil. Root and shoot biomasses were recorded to the nearest 0.1 g after drying for a minimum of 24 h at 80°C.

The effects of density-dependent herbivory on plant biomass, shoot number and height were determined in two experiments using identical protocols. Insect densities were selected along an exponentially increasing scale and chosen to encompass normal field densities from survey data (Cripps *et al.*, 2006). Experiment 1 started on 29 April 2004 with three insect species with ten replicates for each herbivore and density treatment. Treatments were comprised of either *P. cruciferae* (0, 10, 20, 40 and 80 unsexed adults), *P. xylostella* (0, 75, 150, 300 and 900 eggs on foil) or *C. americanus* (0, 1, 2, 4 and 8 adult females). Male *C. americanus* (0, 1, 1, 2 and 4 individuals, respectively) were added to the females to ensure continual fertilization of eggs. Experiment 2 started on 12 August 2004 and tested the impact of *L. hesperus* adults (0, 10, 20, 40 and 80) with five replicates and nymphs (0, 20, 40, 80 and 160) with seven replicates.

Data were analyzed using general linear models for analysis of covariance with herbivore treatments as the fixed factor and position on the greenhouse bench as the random blocking factor. Pre-treatment shoot length was $log_{10}$-transformed and used as a co-variable for biomass and post-treatment shoot lengths. Pre-treatment shoot number was used as a co-variable for post-treatment shoot number. Upon a significant herbivore effect, means were compared using a Tukey Honestly Significantly Different test, and per-capita herbivore effects on shoot length were examined using regression analysis. All analyses were conducted using Minitab® v15 (Minitab Inc., 2006).

## Results

*P. xylostella* was the only herbivore that significantly decreased *L. draba* above-ground ($F_{4,35}$ = 40.09, $P$ < 0.001) and below-ground ($F_{4,35}$ = 47.17, $P$ < 0.001) biomass accumulation (Fig. 1). Damage to plants was extensive at all treatment levels of diamondback moth and resulted in total defoliation of plants at the highest egg density. Caterpillars at the highest density starved to death before pupation, allowing compensatory regrowth of plants before harvest.

Defoliation by *P. xylostella* ($F_{4,35}$ = 6.54, $P$ < 0.001), shot-hole feeding by *P. cruciferae* adults ($F_{4,35}$ = 3.42, $P$ = 0.018) and stem-mining by *C. americanus* larvae ($F_{4,35}$ = 5.24, $P$ = 0.002) significantly decreased maximum shoot elongation (Fig. 2). Sap feeding by *L. hesperus* had no impact on shoot length by either adults ($F_{4,20}$ = 0.51, $P$ = 0.729) or nymphs ($F_{4,28}$ = 0.29, $P$ = 0.884). *C. americanus* had the highest per-capita effect on

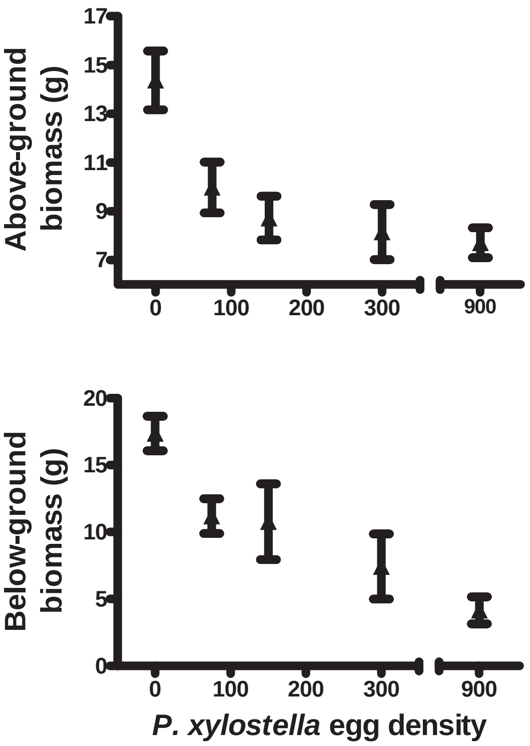


**Figure 1.** Effect of different *Plutella xylostella* egg densities on mean above- and below-ground plant biomass (±95% CI) of *Lepidium draba* after 40 days of larval feeding.

maximum shoot length (–1.25 ± 0.46 cm/female, $P = 0.01$, $r^2 = 0.113$), followed by *P. cruciferae* (–0.151 ± 0.038 cm/adult, $P < 0.001$, $r^2 = 0.234$) and *P. xylostella* (–0.017 ± 0.004 cm/egg, $P < 0.001$, $r^2 = 0.217$) (Fig. 2). None of the herbivore treatments reduced the number of vegetative shoots produced per plant.

*C. americanus* laid eggs in more than 90% of the shoots regardless of female density; however, plant defence responses caused high egg and larval mortality within the stems. Callus tissues were commonly found growing around oviposition holes, eggs and larval mines.

## Discussion

The principal objective of *L. draba* biological control is to reduce the plant's vegetative growth capability, thereby limiting stand dominance and rate of spread of hoary cress. We found that above-ground plant architecture was significantly affected by chewing insects in the defoliating (*P. xylostella*), shot-hole feeding (*P. cruciferae*) and stem-mining niches (*C. americanus*). Our results suggest that the introduction of endophagous stem-miners may impact plant performance even at low densities and should be prioritized as candidates for the biological control of *L. draba*. However, none of the insect species investigated decreased vegetative shoot production of *L. draba*, which is an important determinant of the rate of spread for clones.

Despite the lack of direct impacts on vegetative propagules, above-ground feeding may contribute to the cumulative stress on plants and consequently reduce *L. draba*'s ability to tolerate attack by agents in other niches. Defoliators are not currently being considered for the biological control of *L. draba*, but our data suggest that they can have significant impacts on plant biomass. Shot-holes produced by *P. cruciferae* adults had minimal impact on the performance of bolting plants; we therefore expect that adult feeding by *P. wrasei* will not greatly impair bolting plants either. Adult flea beetle feeding on seedlings and spring rosettes may affect hoary cress population density within patches and sexual reproduction success.

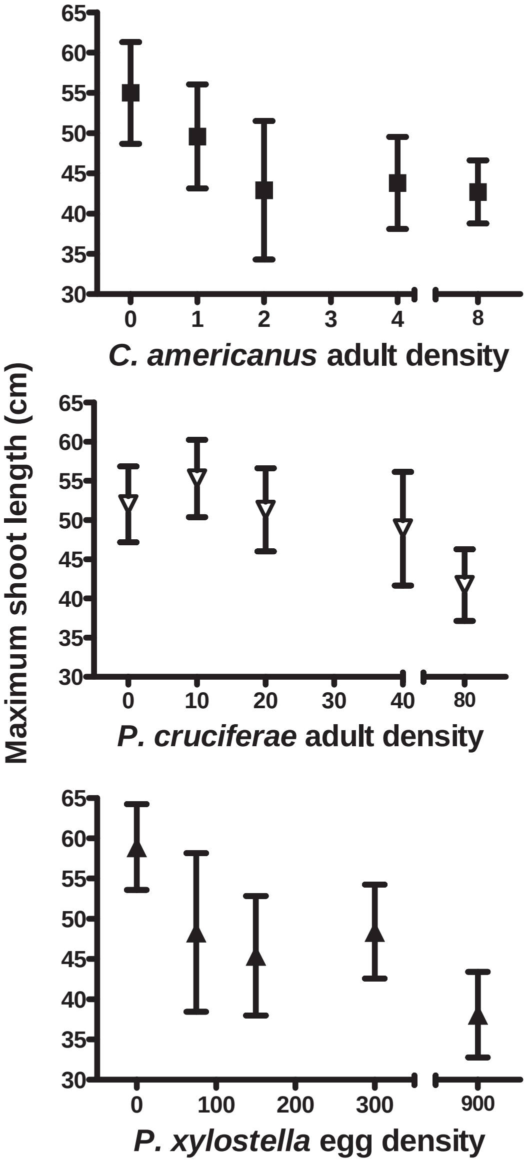


**Figure 2.** Effect of different densities of three surrogate herbivores on maximum shoot length (±95% CI) of *Lepidium draba* after 40 days of feeding.

Faunistic surveys are increasingly being conducted on invasive plants in both their native and introduced ranges (Hinz and Schwarzländer, 2004), providing information on potential surrogate insects. The biogeographic comparison of herbivore faunas from both ranges of *L. draba* by Cripps *et al.* (2006), for instance, provided the opportunity to explore the impact of feeding niches potentially important for biological control of this plant. Another example of the potential uses of surrogate herbivory comes from the search for biological control agents for tropical soda apple, *Solanum viarum* Dunal (Solanaceae), which resulted in the release of the defoliating leaf beetle, *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae) in the USA in 2003 (Cuda *et al.*, 2004). Ten major crop pests that occupy five distinct feeding guilds have been identified on tropical soda apple in the USA (Cuda *et al.*, 2004), and additional feeding guilds are known from the insect pests of potato (*S. tuberosum*; Radcliffe, 1982) and tomato (*S. lycopersicum*; Norris and Kogan, 2005). These pest species could be used as surrogates along with *G. boliviana* to investigate potential interactions between herbivores on *S. viarum* before the introduction of any new biological control agents. Our proposed protocol does not intend to introduce an extra step in the agent selection process but could provide a useful alternative technique to identify and prioritize candidate biological control agents based on empirical efficacy data for agents in different feeding niches.

The greatest limitation for the use of surrogate insects to pre-screen candidate biological control agent efficacy is the availability of specialist feeding niches within the invaded range. Although we identified several above-ground feeding niches with available surrogates, we did not find any appropriate below-ground endophagous feeders or shoot gall formers, both niches for which candidate species have been identified (Fumanal *et al.*, 2004; Cripps *et al.*, 2006). The stem-miner *C. americanus* had high rates of oviposition, but the observed egg and larval mortality may indicate that *L. draba* is not an ideal host plant. Observed effects of *C. americanus* may underestimate the potential impact of better adapted specialist stem-miners such as *C. merkli* and *P. wrasei*, currently being studied at CABI Europe, Switzerland (Cripps *et al.*, 2006). Surrogate herbivores can be used in numerous invasive plant systems beyond relatives of agronomic species, but the best known generalist insect herbivores are crop pests. Several weeds that are unrelated to crops, including *L. draba* (Cripps *et al.*, 2006), are recognized as reservoirs of important economic pests; however, suitable surrogates may not be available for all target weed species and potential biological control niches.

Organizing foreign exploration can take a long time due to logistical, financial, political or safety concerns and may require establishment of new collaborations in areas without a tradition of biological weed control. Furthermore, once candidate insects are found, it takes additional time before mass-rearing techniques are developed to produce sufficient numbers for host-testing and pre-release efficacy testing. Testing the efficacy of surrogate herbivores can provide the opportunity to investigate protocols for screening agents before candidates are identified while simultaneously removing ineffective feeding niches from consideration. The use of surrogate herbivores is therefore not intended to replace efficacy testing but could be used at an early stage of a biological control program to assess the impact of specific feeding niches on the target weed in the invaded range to direct the search for effective candidate agents in the area of origin.

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# The evolutionary history of an invasive species: alligator weed, *Alternanthera philoxeroides*

**A.J. Sosa[1], E. Greizerstein[2], M.V. Cardo[1], M.C. Telesnicki[1] and M.H. Julien[3]**

*Summary*

The eco-evolutionary mechanisms of biological invasions are still not thoroughly understood. Alligator weed, *Alternanthera philoxeroides* (Martius) Gisebach (Amaranthaceae), is a plant native to South America and a weed in Australia and other countries. To better understand its success as an invader, we assessed the morphological and cytogenetic variability of 12 Argentine populations and the cytogenetic variability of seven Australian populations. We found differences in leaf shape (width-to-length ratio) and stem architecture in the Argentine populations, in reproduction (sexual with regular meiosis in two Argentine populations vs completely asexual with irregular meiosis and low pollen viability in all other populations) and ploidy level (tetraploids with sexual reproduction and seed production vs hexaploids with or without sexual reproduction). We propose a hypothesis about the mechanism that drove alligator weed to form highly invasive hybrid populations with vegetative reproduction from diploid ancestors, and we consider the implications for plant–herbivore interactions and biological control of this weed.

***Keywords***: *Alternanthera philoxeroides*, biological invasions, plant–herbivore interactions, hybrids, polyploids.

## Introduction

The identification and characterization of the native range and the centre of origin of a weed are crucial in a biological control program. Alligator weed, *Alternanthera philoxeroides* (Martius) Gisebach (Amaranthaceae), is a target of biological control in Australia. Its native range is southern South America (Argentina, Paraguay, Uruguay and Brazil). In Argentina, the genus *Alternanthera* Forsskal includes 27 species, four of them endemic (Pedersen, 1999), indicating that this is probably its natural area of distribution and perhaps its centre of origin. Alligator weed is represented by two known morphological forms in Argentina, *A. philoxeroides* f. *philoxeroides* (Mart.) Griseb. and *A. philoxeroides* f. *angustifolia* Süssenguth, and a third, intermediate form, all of which were recently associated to a complex of hybrids (Sosa *et al.*, 2004).

*A. philoxeroides* reproduces both sexually and asexually. However, production of viable seeds seems to be restricted to its native range. Understanding why some populations of alligator weed are fertile and others sterile may be important in understanding why this plant is invasive and in the development of management strategies. Factors, both intrinsic, e.g. gametogenesis, and extrinsic, e.g. pollination processes, affect the ability of plants to produce seeds. Seeds and seedlings of both known forms of alligator weed were recorded in the field and in germination trials in the lab (Sosa *et al.*, 2004). Nevertheless, the requirements for successful sexual reproduction, the characterization of hybrids and their role in the invasiveness of the species remain uncertain.

All alligator weed in Argentina propagates vegetatively, and only in particular situations does it also propagate by seed. The reliance on vegetative reproduction could indicate the presence of hybrids in the native range, as in other Amaranthaceae in South America (Greizerstein and Poggio, 1992). Hybrids can be fertile or sterile, depending on the differences between the parental genomes. They can also develop an enhanced

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vegetative reproduction capacity. The strongest evidence for the presence of hybrids can be observed in cytogenetic studies, such as the characterization of meiosis (Mallet, 2005).

For biological control of alligator weed, the flea beetle, *Agasicles hygrophila* Selman and Vogt (Chrysomelidae: Alticinae), the stem borer, *Arcola malloi* (Pastrana) (Lepdidoptera: Pyralidae), and the thrips, *Amynotrips andersoni* O'Neil (Thysanoptera: Phaelothripidae), were released in the USA to control the weed. The flea beetle and the moth were subsequently introduced in Australia (Julien, 1981; Julien and Griffiths, 1999). In spite of their success, there are limitations, mainly concerning terrestrial growth of the weed and the lack of biological control in cooler areas of Australia and the USA (Cofrancesco, 1988; Julien and Bourne, 1988; Julien *et al.*, 1995; Julien and Stanley, 1999).

There are a number of reasons why the flea beetle, currently the most important of the biological control agents, may be restricted in space and habitat. Its life cycle is strongly associated with aquatic conditions, including high humidity. For successful pupation, it requires hollow stems with thin walls (normally found in aquatic growth) rather than thick-walled stems with small cavities (normally terrestrial growth; Vogt, 1973). The flea beetle is also sensitive to low temperatures (Stewart *et al.*, 1996). However, no possible relationship between its performance and plant genetic differences within alligator weed has been considered thus far.

Genetic studies were initiated to understand the morphological variation found in *A. philoxeroides* in its native range and to characterize alligator weed populations in Australia. In addition, a preliminary study was conducted for demographic parameters of the flea beetle under laboratory conditions to evaluate the role of plant ploidy level in the insect's performance.

# Methods and materials

## Morphological studies

Cultures from seedlings and stems from the following different localities in Argentina were cultivated in the greenhouse for 6 months under controlled conditions: 1 – Rt. 11, 22 km SW Reconquista, Santa Fé Province, 29°16′51.3″S, 59°49′12.8″W (from now on called Santa Fé); 2 – Rt. 380, to Lules, Tucumán Province, 26°52′27.5″S, 65°18′25.1″W (Tucumán); 3 – Hurlingham, Buenos Aires Province 34°35′14.2″S, 58°38′24.0″W (Hurlingham) and 4 – Rt.30, 23 km from Tandil, Buenos Aires Province, 37°11′35.5″S, 59°03′29.7″W (Tandil). Twenty-one morphological parameters were measured and analysed using a principal component analysis (PC – ord 4). The variables that explained the greater portion of the variance were analysed using a one-way analysis of variance (ANOVA), and Tukey's honestly significant difference (HSD) test was used to separate the means.

## Cytogenetic studies

The cytogenetic variation of alligator weed was studied to identify hybrid forms in both the native and adventive ranges and to characterize their reproductive status (sexual or asexual). Young flowers were collected into vials with a 6:3:1 solution of 96% ethyl alcohol, chloroform and acetic acid, from 12 sites in Argentina (Fig. 1) and nine in Australia (Fig. 2). The process of meiosis in flower-bud cells was studied. In addition, mitosis was studied in at least 30 cells taken from fine root-tips. Viability of pollen from anthers was estimated using Alexander stain, a stain for chromatin. Unstained pollen grains indicate absence of chromatin and non-viability.

## *A. hygrophila* performance in relation to ploidy levels

Adults of the flea beetle were collected from the field at Hurlingham, near Buenos Aires, and raised in a chamber at 25°C and 12-h light. Ten first-instar larvae were placed in a plastic container (8 cm diameter, 5 cm height) with moistened tissue paper and fed on plants from different alligator weed populations: Santa Fé (hexaploid), Predelta (hexaploid), Hurlingham (hexaploid), Cañuelas (hexaploid) and Tandil (tetraploid). Each treatment was replicated ten times. To estimate performance of the flea beetle on plants from the different localities, survivorship of larvae and immature developmental time were measured. The results were analysed with multivariate ANOVA (MANOVA), and Tukey's HSD test was used to compare means.

# Results

## Morphological studies

The first three axes of the principal component analysis explained 75% of the variance. Populations were ordered according to the variables associated to these axes: shoot diameter, width-to-length ratio of the first leaf and apical angle of the first leaf (Fig. 3). The populations that were more representative of each form (Table 1) were analysed in terms of these three variables. The Cañuelas population closely resembled Tandil population in shoot diameter, but leaves were larger and the width-to-length ratio was intermediate between Hurlingham and Santa Fé. The Predelta population, on the contrary, closely resembled Hurlingham population, although with bigger leaves and shoots.

*A. philoxeroides* f. *angustifolia* differs from *A. philoxeroides* f. *philoxeroides* in the diameter of the stems and in the shape of the leaves. In the laboratory, *A. philoxeroides* f. *philoxeroides* from Tandil (APP in Table 1) had significantly thinner stems (smaller

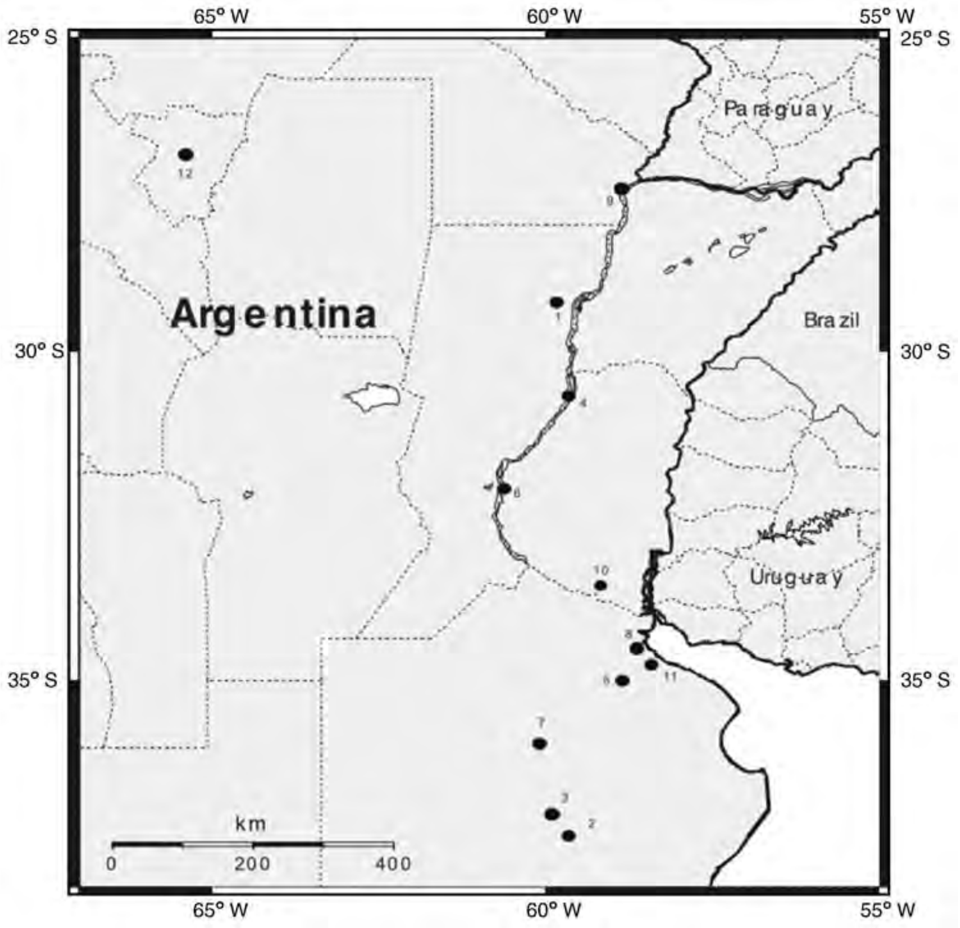


**Figure 1.** The locations of the 12 Argentine populations of *Alternanthera philoxeroides* that were studied. Numbers refer to populations described in Table 2.

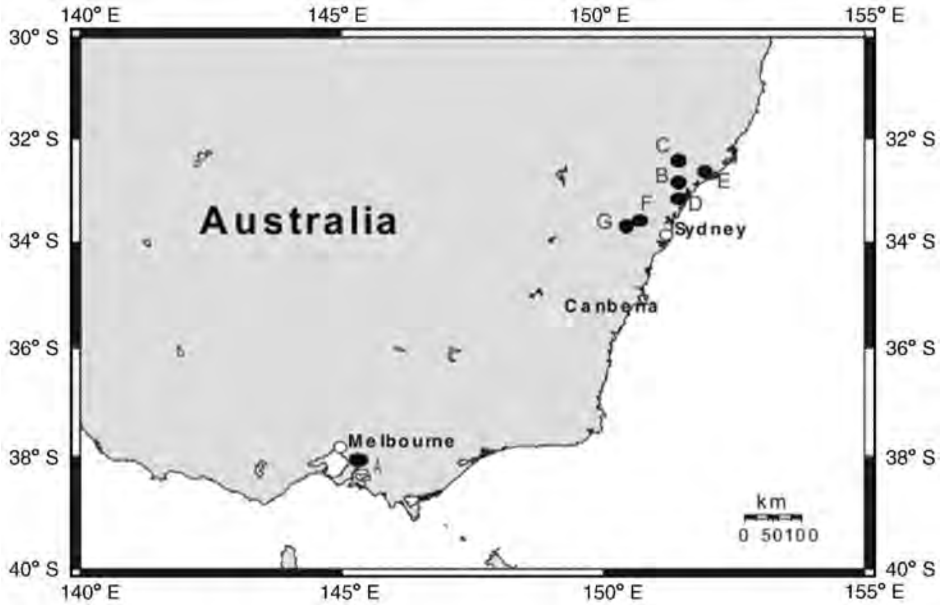


**Figure 2.** The locations of the nine Australian populations of *Alternanthera philoxeroides* that were studied. Letters refer to populations described in Table 2.

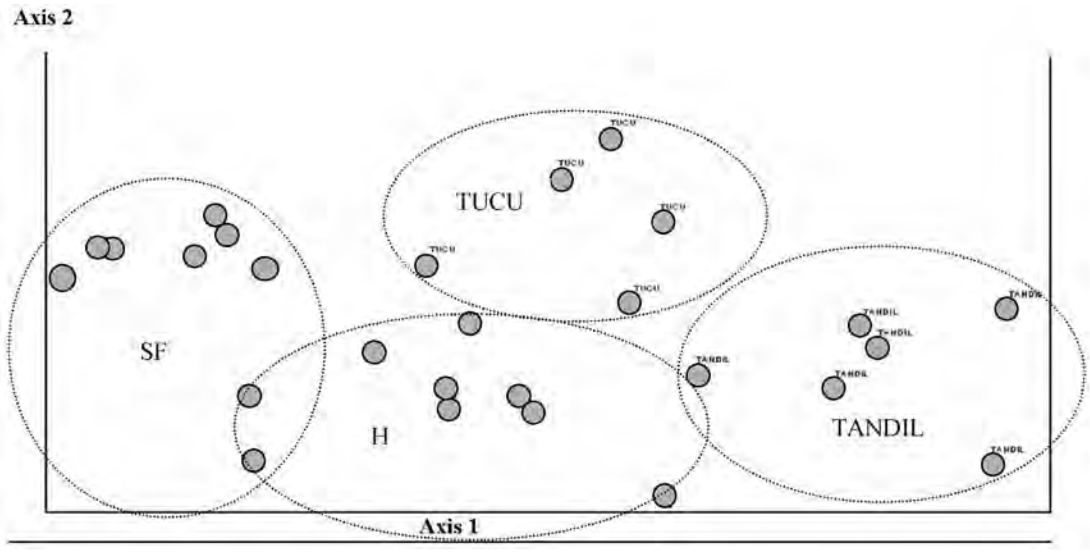


**Figure 3.** Ordination obtained with principal component analysis of four populations of *Alternanthera philoxeroides*; Santa Fé (*SF*), Hurlingham (*H*), Tandil (*Tandil*) and Tucumán (*TUCU*), using 21 morphological variables.

internode diameter), ovate leaf tips (greater leaf apical angle) and shorter leaves (higher values in leaf width-to-length ratio). The *angustifolia* form from Hurlingham (APA in Table 1) significantly differed in its more acute leaf apical angle, longer leaves (lower leaf width-to-length ratio) and broader stems (greater internode diameter). Plants from Santa Fé (intermediate form in Table 1) resembled the *angustifolia* form due to their broad stems, whereas they had similar leaf width-to-length ratio and leaf apical angle to the *philoxeroides* form (Table 1).

## Cytogenetic studies

Alligator weed has small and numerous chromosomes that are difficult to distinguish and count. The results revealed that alligator weed populations in Argentina are composed of a complex of hybrids. Chromosome number differed among populations within the native range (Table 2). Populations from Tandil had 66 chromosomes compared to other populations with higher numbers (approximately 100). In comparison, samples from Santa Fé, Cañuelas, Tucumán and Australia (Table 2) had 'aberrant' meiosis in which several univalent chromosomes were not aligned in the equatorial plate. The resulting cells had different chromosome numbers, approximately 100. Material from Australian populations could not be analysed for chromosome number because root-tips were in an advanced state of cell division at the time of the study.

It is interesting to note that *Alternanthera aquatica* Chod., a species closely related to alligator weed, also had 66 chromosomes. This is the first report of a chromosome number for this plant species.

Pollen staining confirmed low viability of pollen grains, suggesting hybrid forms of *A. philoxeroides*. Pollen viability from Tandil and Predelta was higher than in Santa Fé, Cañuelas, Hurlingham or Australia (Table 2).

## *A. hygrophila* performance in relation to ploidy levels

Preliminary results show that host genetic variability affects the performance of *A. hygrophila* (MANOVA: Wilks lambda = 0.63, $P$ = 0.008). Differences were found in immature survivorship (Fig. 4, ANOVA: $F_{4, 44}$ = 4.07, $P$ = 0.006), which was higher in plants from Hurlingham (0.77 ± 0.03) than in those from either Santa Fé (0.44 ± 0.07) or Predelta (0.49 ± 0.08),

**Table 1.** Morphometric data for laboratory-grown *Alternanthera philoxeroides* from three locations in Argentina. *APP*, *A. philoxeroides* f. *philoxeroides*; *APA*, *A. philoxeroides* f. *angustifolia*; *AP?*, an intermediate form. Means and standard errors are shown. Means *within a column followed by different letters* are significantly different ($P$ < 0.05; ANOVA, Tukey post hoc multiple comparisons).

| Form | Collecting site | Internode diameter (mm) | Leaf length to width ratio | Leaf apical angle (°) |
|---|---|---|---|---|
| APP | Tandil, *n* = 10 | 1.95 ± 1.11a | 0.40 ± 0.07a | 137.33 ± 43.16a |
| AP? | Santa Fé, *n* = 10 | 2.84 ± 0.77 b | 0.42 ± 0.12a | 105.56 ± 31.00a |
| APA | Hurlingham, *n* = 10 | 3.16 ± 0.48b | 0.24 ± 0.05b | 68.83 ± 14.45b |

**Table 2.** Results of cytogenetic studies on 12 Argentine populations of *Alternanthera A. philoxeroides*. The *dash* (–) indicates that data is not yet available. The *numbers in the first column* relate to locations in Figs. 1 and 2.

| Population | Number of individuals | Form | Chromosome number 2n | Pollen stainability (%) | Observations on pollen grains | Fruits |
|---|---|---|---|---|---|---|
| **Argentina populations** | | | | | | |
| Reconquista, Santa Fé (1) | 5 | Intermediate[a] | Approximately 100 | 50 | Different size (aneuploid) | No |
| Tandil, Buenos Aires (2) | 6 | *philoxeroides* | 66 | 95 | Normal morphology and size | Yes |
| Tandil, Buenos Aires (3) | 4 | *philoxeroides* | 66 | 95 | Normal morphology and size | Yes |
| La Paz, Entre Ríos (4) | 5 | *angustifolia* | | 61 | Different size (aneuploid) | No |
| Cañuelas, Buenos Aires (5) | 5 | *angustifolia* | *ca.* 100 | 0 | | Yes |
| Predelta, Entre Ríos (6) | 4 | *angustifolia* | *ca.* 100 | 94 | Normal morphology and size | No |
| Cazón, Buenos Aires (7) | 4 | *intermediate | | 65 | Different size (aneuploid) | No |
| Hurlingham (8) | 5 | *angustifolia* | | 0 | | Yes? |
| Chaco (9) | 5 | *A. aquatica?* | 66 | 15 | Different size (aneuploid) | No |
| Paranacito (10) | 5 | *A. aquatica?* | | 0 | – | No |
| La Plata (11) | 5 | *intermediate | 66 | – | – | Yes |
| Tucumán (12) | 5 | *angustifolia* | 100 | – | – | No |
| **Australian populations** | | | | | | |
| Hunter Valley, NSW (A) | 3 | *angustifolia* | – | 5 | Different size (aneuploid) | |
| Dandenong, Victoria (B) | 2 | *angustifolia* | – | 8 | Different size (aneuploid) | |
| Kaotara, NSW (C) | 4 | *angustifolia* | – | – | | |
| Maitland East, NSW (D) | 3 | *angustifolia* | – | 8 | Different size (aneuploid) | |
| Wallsend, NSW (E) | 4 | *angustifolia* | – | – | | |
| Oakville, NSW (F) | 5 | *angustifolia* | – | 7 | Different size (aneuploidy) | |
| Richmond, NSW (G) | 3 | *angustifolia* | – | 6 | | |

[a] Intermediate: A form that appears to be intermediate between the two forms *angustifolia* and *philoxeroides* (see Table 1).

but showed no difference from plants from Tandil (0.57 ± 0.06) or Cañuelas (0.57 ± 0.06).

However, developmental time (in days) of different hybrids were not different (Santa Fé 22.2 ± 0.8; Predelta 21.1 ± 1.1; Hurlingham 21.2 ± 0.6; Tandil 23.1 ± 0.9; Cañuelas 20.4 ± 0.5; ANOVA: $F_{4, 44} = 1.85$, $P = 0.136$).

## Discussion

Irregularities in meiosis division are associated with hybrid organisms (Mallet, 2005). Irregularities observed through cytogenetic analysis, and subsequent correlation with pollen staining strongly suggests that the entity *A. philoxeroides* is a complex of hybrids. Additional chromosomes (through hybridization) could be beneficial for these plants (Levin, 2002), particularly as they do not depend on sexual reproduction. New polyploids may possess novel physiological, ecological or phenological characteristics that allow them to colonize new niches, and they may be wholly or partially reproductively isolated from their diploid progenitors (Ramsey and Schemske, 1998).

Based on our results and knowing that in the Gomphreninae tribe the basic chromosome number is $x$ = 16–17 (Okada *et al.*, 1985), we propose a hypothetical model showing the evolution of *A. philoxeroides* in its native area (Fig. 5). Diploid ancestors gave origin to

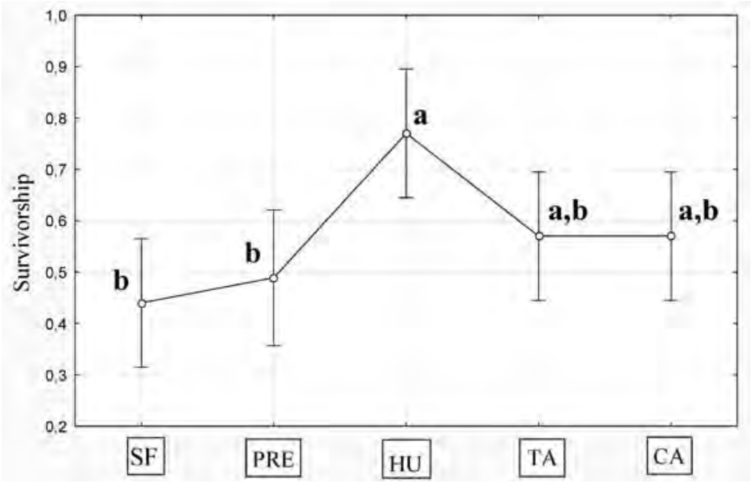


**Figure 4.** Survivorship of larvae of *Alternanthera hygrophila* on five populations of *A. philoxeroides* from Argentina; Santa Fé (*SF*), Predelta (*PRE*), Hurlingham (*HU*), Tandil (*TA*) and Cañuelas (*CA*). Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences (ANOVA *P* < 0.05).

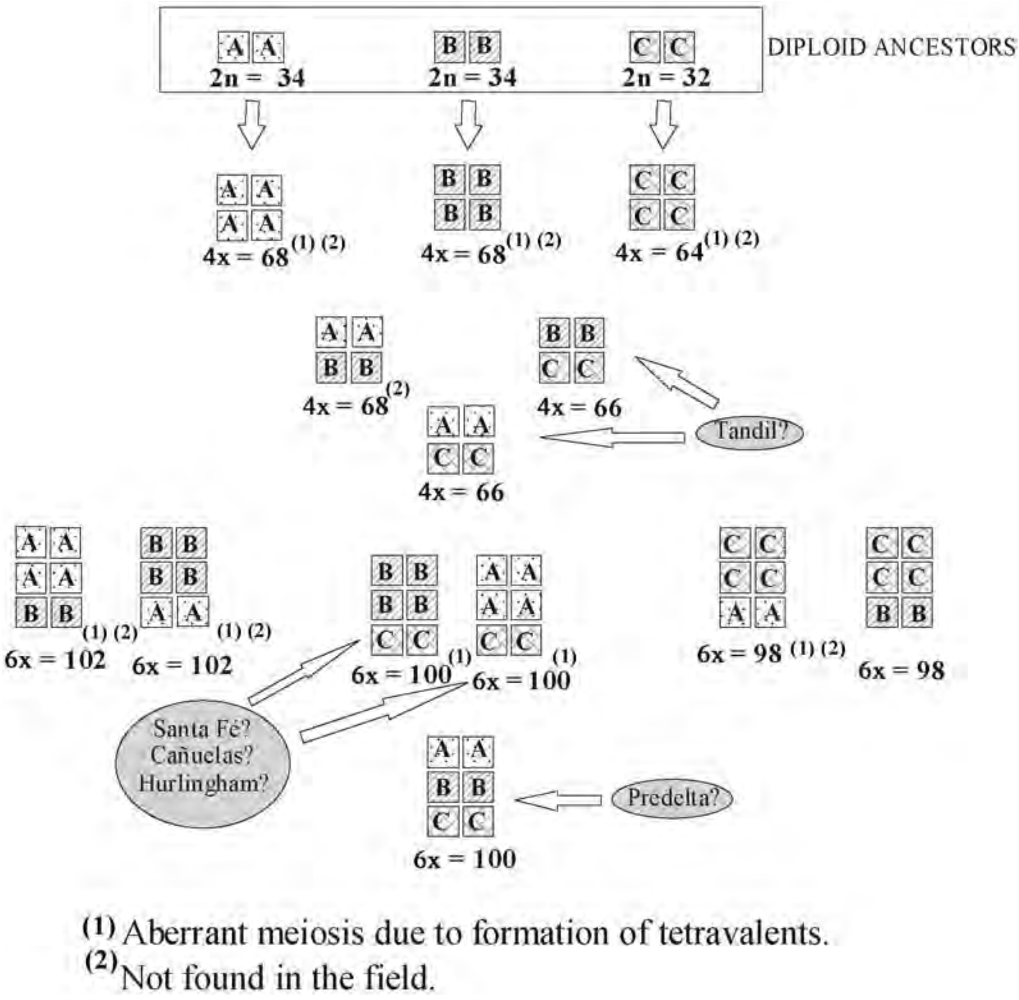


**Figure 5.** Hypothetical hybridization model of *Alternanthera philoxeroides* in Argentina.

auto- and allotetraploids, the latter being represented by the Tandil population. Due to a lack of reduction in meiosis I, these subsequently produced two different types of hexaploids, one with aberrant meiosis (Santa Fé–Cañuelas–Hurlingham) and another with normal meiosis and sexual reproduction (Predelta). However, it is still uncertain which parents gave rise to the alligator weed hybrids. To assess this issue, the genomic in situ hybridization technique will be used in the future to determine the origins of the different chromosomes in each population. This technique is used with the combination of molecular tools to study the evolutionary aspects and the role of polyploids in other biological invasions (Fehrer *et al.*, 2007). For the alligator weed, they should provide a more complete picture of its evolutionary history.

As the only cytotype of *A. philoxeroides* found in Australia is a hexaploid, we suggest that its invasive capability could be attributed to traits from one of the native range hexaploid (hybrid) populations and that these traits were gained before invasion. As previously documented for other weeds (Lee, 2002), alligator weed shows positive effects of hybridization on invasibility, such as faster growth, greater size and increased aggression (Okada *et al.*, 1985; Alonso and Okada, 1996). In addition, alligator weed plants growing in aquatic and terrestrial environments are morphologically different, and this might be due to genotype × environment interactions.

The alligator weed flea beetle that was released in the USA and Australia was collected in Buenos Aires Province in Argentina and in Montevideo, Uruguay (Coulson, 1977). The insects used in the present study were collected in Hurlingham. As it is highly probable that these populations have the same origin, the use of insects from Hurlingham for plant–herbivore interaction studies may validate comparisons between the outcome of our experiments and the behaviour of *A. hygrophila* in the adventive range. In our experiments, higher survivorship on Hurlingham plants could be explained by differences in the genotypes or by plant maternal effects. This warrants further study, and a current experiment is testing for differences in fertility and fecundity.

The presence of different cytotypes of alligator weed should be considered when explaining the lack of success of the flea beetle in controlling the weed in cool areas, particularly in the USA, where the existence of distinct alligator weed biotypes has been confirmed (Kay and Haller, 1982).

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# Landscape genetics and climatic associations of flea beetle lineages and implications for biological control of tansy ragwort

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*Summary*

While *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae) has shown the best results for biocontrol of tansy ragwort, *Senecio jacobaea* L. (Asteraceae), the precise effects of this flea beetle on tansy ragwort distribution and abundance are confused by the introduction in North America of two distinct strains, of Italian and Swiss origin. Beetles of the two biotypes differ in their phenology, while hybrids of the two strains show life-cycle characteristics different from either parental strain. However, it is not known which biotype(s) currently provides control of tansy ragwort infestations or whether both biotypes or hybrids of the two have been established in the USA. Moreover, mixed populations may exhibit lower efficiency in controlling the target weed relative to pure strains, and specificity for the invasive host may be compromised. In this study, molecular markers were employed to distinguish between biotypes of *L. jacobaeae*. Analysis of mitochondrial sequence of specimens from Switzerland, Oregon and California revealed higher than expected sequence variation, within and between strains. Despite the high levels of polymorphism, cladistic analysis did not show distinct separation of strains into well-defined clades. These results indicate that Swiss *L. jacobaeae* beetles may have established in North America in contrast with the general assumptions. As the revealed mitochondrial DNA (mtDNA) polymorphisms themselves will not identify populations, we are now employing nuclear markers to characterize North American populations, their ancestry and their association with certain climatic regions. The goal is to match the most effective genotypes of *L. jacobaeae* with new tansy ragwort infestations east of the Cascade mountain range.

***Keywords***: COI, sequence variation, hybrid strain, insect biotypes.

## Introduction

Tansy ragwort is one of the fastest spreading invasive plants in the western USA since its introduction in 1922 (McEvoy, 1984). It is particularly prevalent in Washington, California and Oregon, has spread recently into Montana and Idaho and is listed as a noxious weed in eight states. West of the Cascade Mountain range, tansy ragwort infestations have been most effectively controlled with biological control agents (McEvoy *et al.*, 1991). Tansy ragwort biomass was reduced by 93% in western Oregon (Coombs *et al.*, 1996) and by 99% in sites in northern California (Hawkes and Johnson, 1978) after the introduction of three biological control agents, but the success is primarily attributed to *Longitarsus jacobaeae* Waterhouse (McEvoy *et al.*, 1991). Although *L. jacobaeae* is the most effective biocontrol agent, quantifying the effects of this flea beetle on tansy ragwort distribution and abundance is difficult due to the introduction of two distinct biotypes, or strains, of Italian and Swiss origin in North America (Frick, 1971; Frick and Johnson, 1973).

Beetles of the two biotypes differ in their phenology and environmental requirements, and hybrids of the two biotypes show phenologies different from that of either

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parental strain (Frick, 1971; Frick and Johnson, 1973). While the establishment of the Italian biotype of *L. jacobaeae* is widely assumed in western coastal areas, the fate of the originally released Swiss beetles in Del Norte County, California (Frick, 1970) is not known. Thus, it is not clear which biotype(s) are currently providing effective control of coastal infestations of tansy ragwort or whether hybrids of these biotypes have been established in the USA. Beetles derived from these North American coastal populations have failed to establish in areas east of the Cascade Mountain range, which are characterized by a continental climate with cold winters (Turner and McEvoy, 1995). Mixed populations might be less effective in controlling tansy ragwort infestations, and hybrids may differ in their host specificity, placing native confamilial plants at risk (Hoffman *et al*., 2002). Consequently, it is a matter of great importance to determine the origins and composition of extant populations in North America.

Comparison of the genetic composition of extant *L. jacobaeae* populations in North America with European source populations will not only indicate the origins of North American populations but also reveal patterns of hybridization and climatic associations of *L. jacobaeae* lineages. In this study, we investigated two introduced North American populations and one Swiss population of *L. jacobaeae* to assess whether mtDNA sequences can reveal sufficient genetic variation and differentiation between strains that may allow development of molecular markers that can distinguish biotypes of the flea beetle.

# Methods and materials

## DNA extraction

DNA was extracted from 50 individuals using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA), following the manufacturer's protocol. Twenty-two individuals were collected from Mettembert, Switzerland (47°24′N, 7°20′E), 14 specimens were collected both from Salem, OR (44°93′N, 122°99′W) and from Crescent City, CA (41°44′N, 124°08′W).

## DNA amplification

Polymerase chain reaction (PCR) was performed with general-purpose insect-derived primers that are known to amplify a fragment of the coleopteran mtDNA genome (Szalanski and Owens, 2003):

C1-J-2797: 5′-CCTCGACGTTATTCAGATTACC-3′
C2-N-3400: 5′-TCAATATCATTGATGACCAAT-3′

These primers amplify the 3′ end of the cytochrome oxidase I gene, the transfer RNA for leucine, and the 5′ end of cytochrome oxidase II gene. Each reaction was carried out in 20 μl reaction volume, containing 1× Colorless GoTaq buffer pH 8.5, 1.5 mM $MgCl_2$, 1 U of GoTaq DNA polymerase (Promega), 10 pmol of each primer, 0.2 mM deoxyribonucleotide triphosphate and approximately 25 ng DNA. Thermal cycling conditions were 3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 20 s at 51°C, 50 s at 72°C, with a final step of 3 min at 72°C.

## DNA sequencing and analysis

PCR products were prepared for sequencing using ExoSAPit (GE Healthcare Corp., Piscataway, NJ), following the manufacturer's protocol. Cleaned samples were sequenced with Big Dye version 3.1 following the manufacturer's protocol and the reactions run on an Applied Biosystems 3130*xl* automated sequencer. Sequences were edited and aligned with Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI). Parsimony analysis was carried out using PAUP 4.0b10 for Macintosh (Swofford, 2002). For the tree shown in Fig. 1, *Apthona cyparissiae* Koch was used as an outgroup to root the tree (accession number gi88810025), incorporating 541 bp of sequence data. Thirty-two sites were parsimony-informative, including 50 haplotypes from North American and Swiss populations. Search was full heuristic, with branch swapping [tree bisection-reconnection (TBR)] and gaps treated as missing data. To gauge the reliability of the observed topology, we did a further round of bootstrap analysis using a different outgroup, *Diabrotica barberi* Smith and Lawrence (accession number gi11275649), and a reduced number of taxa. For the bootstrap analysis, we used 27 haplotype samples representing all the major branches of the tree shown in Fig. 1, incorporating 588 bp of sequence data, of which 13 sites were parsimony-informative. Search was full heuristic with branch swapping, gaps were treated as missing data and 100 replicates were performed.

# Results

The *L. jacobaeae* sequences revealed an unexpectedly high level of polymorphism. Ninety-eight polymorphic sites were detected within the analysed 541 bp region, of which 32 were parsimony-informative. The sequences were obtained from 50 individuals from three populations (two North American and one Swiss). By way of comparison, sequencing the same region from 22 individuals of the southern corn rootworm, *Diabrotica undecimpunctata*, revealed two haplotypes, differentiated by a single nucleotide polymorphism (Szalanski and Owens, 2003). High levels of polymorphism, notwithstanding, parsimony analysis of the unique haplotypes did not show clustering of strains into well-defined clades. Results of this analysis are shown in Fig. 1. To test the reliability of the topology we obtained in our parsimony analysis, we carried out bootstrap analysis (Hillis and Bull, 1993). Bootstrap analysis indicated a 50% majority-rule consensus tree in which

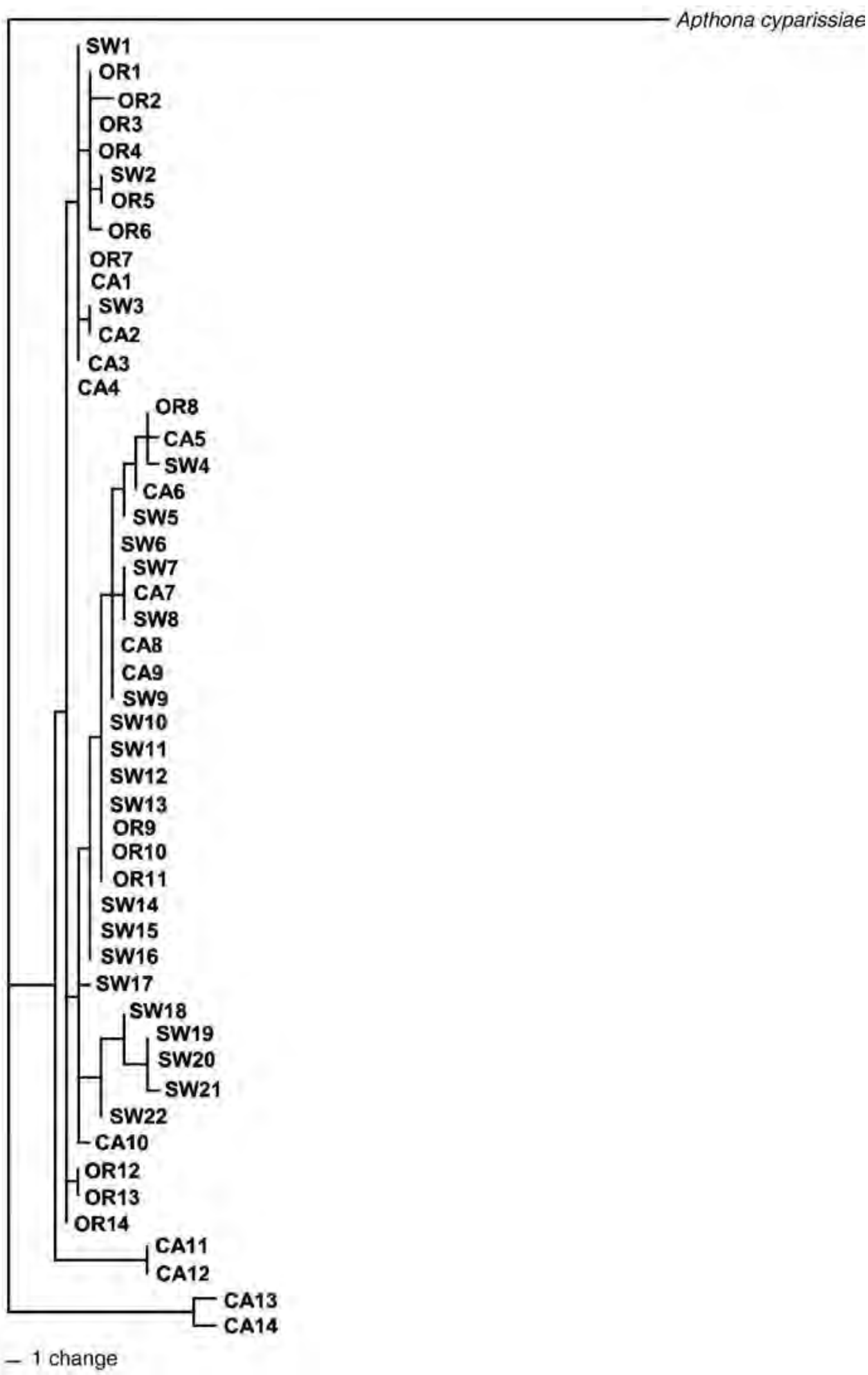


**Figure 1.** Rooted parsimony tree constructed in PAUP using heuristic search with branch swapping (TBR) and gaps treated as missing data. Branch lengths as shown. *Longitarsus jacobaeae* originating from Switzerland (*SW1–22*), Oregon (*OR1–14*) and California (*CA1–14*).

all North American and Swiss samples cluster in a simple polytomy (Fig. 2).

## Discussion

Our results show that there is substantial sequence variation in the mitochondrial genome of *L. jacobaeae*, both within and between strains, suggesting that further investigation of other regions will yield genetic markers indicative of strain type. Both Italian- and Swiss-strain beetles were originally released in California, but only the establishment of the Italian biotype was confirmed, and it is now widely accepted that all beetles distributed along the west coast are of Italian origin (Turner and McEvoy, 1995). According to this assumption, the CA and OR populations sampled for this study

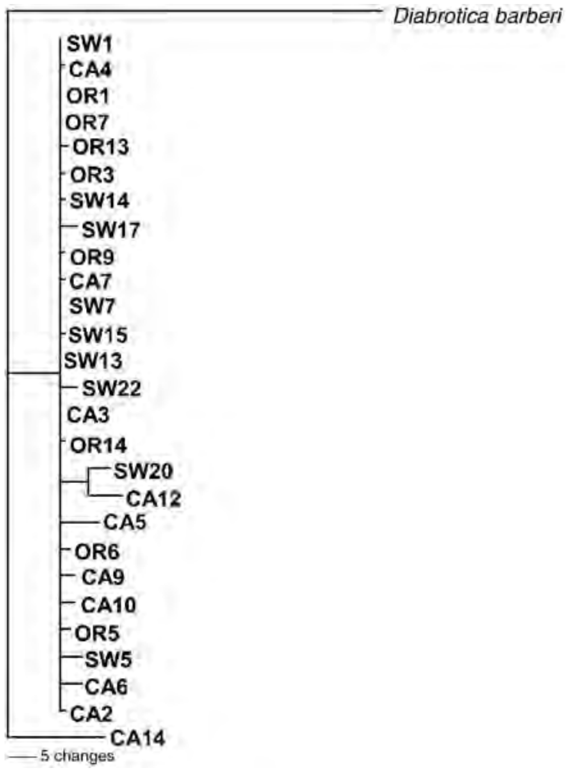


**Figure 2.** Bootstrap analysis conducted with full heuristic search with branch swapping, gaps treated as missing data and 100 replicates performed. Branch lengths as shown. *Longitarsus jacobaeae* originating from Switzerland (*SW1–22*), Oregon (*OR1–14*) and California (*CA1–14*).

were derived from the Italian strain. Our results are surprising in light of this general assumption. Because of the strikingly high level of observed polymorphism, a trend would be expected for the clustering of haplotypes if the sampled beetles represent two distinct biotypes, such as the Swiss and Italian. One possible explanation for the lack of segregation of strains may be that Swiss beetles from the original releases have established in California, and either significant hybridization or introgression occurred between the Italian and Swiss beetles. This seems a plausible scenario, as *L. jacobaeae* used for this analysis was collected in Del Norte County in California where Swiss beetles were initially released and Californian populations later provided the source populations for the redistribution of flea beetles along the west coast.

However, definitive conclusions cannot be drawn from this sampling because it lacks representative beetles of known Italian origin. Therefore, we are making efforts to obtain beetles from Italy. In addition, we will be employing nuclear markers to assess the ancestry of North American populations and their association with certain climatic regions. The goal is to match the most effective genotypes of *L. jacobaeae* with new tansy ragwort infestations east of the Cascade Mountain range.

## Acknowledgements

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# Genetic characterization of the whitetop collar gall weevil, *Ceutorhynchus assimilis*, enhances its potential as biological control agent

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In the field of biological control, it is becoming clear that genotypes of invasive weeds vary in their susceptibility to natural enemies and that genotypes of actual or candidate biological control agents vary in their ability to control different genotypes of target weeds, influencing the level of total control achieved. Few studies attempt to both pinpoint the area of provenance of the invasive weed in the introduced range and search for matching genetic relationships between the target weed and the herbivore before selection of biological control agent. The case of hoary cress, *Lepidium draba* (Brassicaceae, native to Eurasia), which is one of the most invasive noxious weeds in North American rangelands and croplands, and one of its natural enemies, a collar gall weevil, *Ceutorhynchus assimilis* (Coleoptera: Curculionidae), explicitly illustrates the benefit of using evolutionary knowledge to refine efficacy and safety of biological control. Within the geographic distribution of the phytophagous weevil, genetic analysis uncovered several morphocryptic genetic lineages including one race specific to *L. draba* with regard to the larval development and restricted to southern France and northern Italy. Crossing experiments were carried out to assess the level of reproductive isolation of these lineages. Concomitantly, a phylogeography study of the weed in its native range gave evidence of a cluster of haplotypes originating from the same region as the *L. draba* specific race found in the weevil.

# Pinpointing the origin of North American invasive *Vincetoxicum* spp. using phylogeographical markers

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Three European species of swallow-worts belonging to the Apocynaceae family are established in North America. *Vincetoxicum nigrum* (L.) Moench (black swallow-wort) and *Vincetoxicum rossicum* (Kleo.) Barb. (pale swallow-wort) are both highly invasive in natural areas, abandoned pastures and rural sites. *Vincetoxicum hirundinaria* Medik. (white swallow-wort) occurs sparsely in the Northeast as a horticultural escape. As current control measures for the swallow-worts are unable to alleviate their weedy impact and because of the numerous natural enemies associated with *Vincetoxicum* sp. in Europe, classical biological control of swallow-worts in North America is being considered. Ascertaining the insect fauna of *Vincetoxicum* species in Eastern Europe and western Russia is confounded by problems in target plant taxonomy at both species and genus levels. Tracing the origins of invasive weeds and knowing the levels of genetic variation relative to the native range seems to be increasingly important for conducting rigorous specificity tests in the time frame of a biological control programme. Currently, nothing is known about the genetic relationships between native and introduced populations of these target weeds. More importantly, with the complexity of the genus, the present taxonomic identity of individuals is questionable. In collaboration with national research agencies, plant material of these species is being collected from populations in native and introduced ranges. Phylogeography is being explored using chloroplast DNA sequences in combination with ploidy determination and first data presented in this paper.

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# Population genetics of invasive North American diffuse and spotted knapweed (*Centaurea diffusa* and *C. stoebe*)

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Knowing the possible origins of invasive weeds, whether multiple introductions have occurred, and levels of genetic variation relative to the native range is vital to conducting rigorous tests of several hypotheses that underlie classical biological control. We explore the population genetics of two Eurasian species that are invasive in North America, *Centaurea diffusa* and *Centaurea stoebe*, using variable chloroplast DNA (cpDNA) sequences and microsatellite loci. *C. diffusa* has lower haplotype diversity and cpDNA allelic richness in the introduced range relative to the native range, suggesting that the introduction imposed a bottleneck in population size. However, variation at microsatellite loci does not differ, and the data suggest a minimum of two introductions of *C. diffusa*. Three of the haplotypes of *C. stoebe* found in North America match haplotypes in species other than *C. stoebe* from the native range, suggesting the possibility of cryptic invasions. Additionally, *C. diffusa* and *C. stoebe* share several cpDNA haplotypes, including their most common haplotype, and they share most microsatellite alleles. This suggests ongoing hybridization between the species or incomplete segregation of alleles. These data can guide further exploration for the origins of these species and point out locations within the introduced range with unique and diverse genetic makeup.

# Morphological and genetic methods to differentiate and track strains of *Phoma clematidina* on *Clematis* in New Zealand

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A highly pathogenic strain of the leaf pathogen, *Phoma clematidina* (Thüm.) Boerema, has been deliberately introduced to New Zealand from North America for biocontrol of old man's beard, *Clematis vitalba* L. However, the disease levels of this biocontrol agent have been inconsistent, and it is sometimes present as a symptomless endophyte. Local strains of *P. clematidina* that are mildly or non-pathogenic on *C. vitalba* were present in New Zealand on *C. vitalba* and native *Clematis* species before initiation of the biological control programme. An understanding of how the introduced virulent biocontrol strain is interacting with the avirulent endemic strains, and how this interaction is impacting on the pathogenicity and disease expression on *C. vitalba* is critical to fully evaluate biocontrol efficacy of the disease. We are using morphological and genetic methods to differentiate local strains of *P. clematidina* from the exotic biocontrol strain. These are also being used to determine the distribution, pathogenicity and host specificity of the different pathogenic strains present in New Zealand. 'Marked' *P. clematidina* strains are being developed to track the fungus in the field to further understand the epidemiology of *P. clematidina* leaf disease on *Clematis*.

# Polyploidy, life cycle, herbivory and invasion success: work on *Centaurea maculosa*

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The knapweed, *Centaurea maculosa*, has been introduced from Europe (EU) into North America (NA) during the late 19th century, where it has become a prominent rangeland weed. Flow cytometry studies of populations sampled in EU and NA revealed diploid (2x) and tetraploid (4x), as well as few mixed populations in EU but, so far, only 4x populations in NA. Field observations suggest that 2x populations are predominantly monocarpic and 4x populations polycarpic. Age structure using herb chronology will be presented for various populations. In the greenhouse, we are growing plants of 77 native EU populations, both 2x and 4x, and of 23 invasive 4x NA populations, conducting performance tests with specialist and generalist herbivores and analyzing defence traits. We specifically explore the link between ploidal level, life history traits, phenotypic plasticity and reproductive strategy to investigate trade-offs with defence traits. To test if a polycarpic habit has been negatively selected by specialist herbivores in the native but positively in the introduced range, where specialist herbivores are absent, we started a 2x vs 4x competition experiments in the presence and absence of herbivores. The results will be integrated with information from niche modelling and community invasibility experiments.

# Use of morphometrics and multivariate analysis for classification of *Diorhabda* ecotypes from the old world

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Six years of data have shown high potential of *Diorhabda elongata* as an effective biological control of *Tamarix* spp. in some regions of the USA. There is evidence that five ecotypes may represent different sibling species. Consequently, taxonomic studies of the saltcedar beetle are critical in *Tamarix* control programs. In addition, there is disagreement among taxonomists about the existence and number of *D. elongata* sibling species. Five genitalic ecotypes based on morphology of the genitalia are reported: elongata, carinata, sublineata, carinulata and meridionalis. These ecotypes may be suitable for control of *Tamarix* in differing biogeographic areas of the western USA. We developed a classification system of *Diorhabda* ecotypes based upon measurements of both genitalic and external structures using a combination of factor and cluster analyses. The first factor associated with 59% of the variability is explained by external body parts; the other four factors are associated with genitalic measurements and together explained 34.24% of the data variability. The cluster analysis was able to reproduce a good separation of the 85 specimens into five ecotypes. A dendrogram constructed from the analysis shows the highest affinity between the carinata and sublineata morphotypes and the highest difference between elongata and the rest of ecotypes.

# Why are there no species-specific natural enemies for giant hogweed?

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Based on surveys for and laboratory studies of the insect herbivores and fungal pathogens recorded from giant hogweed, *Heracleum mantegazzianum* Sommier & Levier (Apiaceae) in the Caucasus as its native range, we assess the potential for classical biological control of giant hogweed in Europe. Surveys revealed a guild of natural enemies, arthropods and pathogens, associated with the target plant and other *Heracleum* spp. in the western Caucasus Mountains. However, none of the evaluated insects and pathogens was considered to be suitably host-specific for introduction into Europe. A hypothesis is proposed to explain the absence of monospecific natural enemies of giant hogweed in the Caucasus, based on the dynamic and interactive evolution of populations of closely related and hybridizing species of *Heracleum* spp. in this mountain range over successive glaciation events during the Pleistocene.

# Specificity and plant host phenology: the case of *Gephyraulus raphanistri* (Diptera: Cecidomyiidae)

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Wild radish (*Raphanus raphanistrum* L.) is one of the most important weeds of crops in southern Australia. The potential for classical biological control of this weed was investigated, and recent confirmed host records show that the flower gall midge, *Gephyraulus raphanistri*, is restricted to *R. raphanistrum* throughout Europe. *G. raphanistri* has never been confirmed from Canola in Europe, where 3 million hectares are grown each year. Field host specificity testing *G. raphanistri* by manipulating host plant phenology of actual and potential hosts in the genera *Raphanus* and *Brassica* revealed that no host plant preference was observed. All tested species, *Raphanus raphanistrum raphanistrum* (wild radish), *Raphanus raphanistrum landra* (coastal wild radish), *Raphanus sativus* (radish) and *Brassica napus* (an oilseed rape cultivar) were resynchronized for initial flowering to the natural *R. raphanistrum landra* plants hosting a natural population of *G. raphanistri*. The high field host specificity observed in this gall midge in Europe is driven by synchrony of oviposition and flower availability. When phenologically resynchronized, Canola was an equally acceptable host in the field for oviposition and larval development. In Australia, the new environment might generate new phenological conditions and thus significantly increase the risk associated with this midge as a biological control agent.

# Comparative invasion histories of Australians invading South Africa

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An increasing number of studies are exploring the phylogeography of invasive alien species and how this may impact the success rate of biological control programs. We are in the first year of a comparative study looking at plants native to Australia and invasive in South Africa (and vice versa), focussing on acacias in particular. We believe that, by comparing species, we can gain general insights that are of value both to direct management and to our broader understanding of invasion biology. We also contend that the collection and curation of plant material samples suitable for genetic analysis should form part of any biological control survey. We are keen to hear comments, share experiences and establish collaborations.

Theme 7:

# Opportunities and Constraints for the Biological Control of Weeds in Europe

## Session Chair: Paul Hatcher

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Keynote Presenter

# Opportunities and constraints for the biological control of weeds in Europe

**M. Vurro[1] and H.C. Evans[2]**

*Summary*

Although there has been increasing interest in Europe over the last few decades in trying to harness the potential of biological control as a management tool for weeds, securing funding for projects continues to be problematic whilst the successes have been limited. The incentives to use alternative technology are based on a number of interrelated factors, not least the dramatic rise in popularity of organic products (linked to increasing environmental awareness) and a powerful anti-GMO lobby. Combine this with new legislation to remove some chemical herbicides from the market or to restrict their usage, as well as soaring development costs for new products, and the opportunities for biological control have never been better. Here, we analyse the reasons for the limited uptake, and the challenges or constraints facing weed biocontrol from two different approaches: classical and inundative. For classical biological control against invasive alien weeds, Europe has lagged behind other continents (e.g. Australasia, North America) to the point that there have been no introductions thus far, whilst those that are in the pipeline still need to clear considerable legislative hurdles. These issues are highlighted for past and ongoing projects. For inundative biological control against indigenous or naturalized weeds—in this case, the use of products based on plant pathogens (bioherbicides)—the constraints are largely technological and commercial rather than bureaucratic: e.g. stability and efficacy; costs of production and registration; and limited markets. Opportunities to support the study and use of mycoherbicides, and strategies to overcome these constraints—improving production, formulation and application systems; genetic enhancement; synergistic mixtures of agents/metabolites—are discussed.

***Keywords***: bioherbicides, classical biological control, environmental weeds.

## Introduction

In a review of the biological control of weeds and its prospect in Europe, Schroeder and Müller-Schärer (1995) stated optimistically that: 'Although biological weed control so far [has] received little attention in Europe, more recent developments indicate that this may change in the near future'. These developments included the commercialization of, and increasing potential for, mycoherbicide use, particularly in agricultural systems in North America (Charudattan, 1991; Smith, 1991). In the intervening decade or so, has this optimism been realized? Certainly, the odds should have moved favourably in this direction, not least because of the current awareness of environmental issues in Europe by the public and politicians alike, leading to: a dramatic rise in the popularity of organic products; a powerful anti-GM lobby, negating the use of herbicide-resistant crops; and new legislation to remove chemical herbicides from the market, or to restrict their usage.

Here, this question is addressed, together with an assessment of the opportunities and constraints for biological control of weeds in Europe.

### Classical

**Crispy concepts, soggy concerns:** Classical (or inoculative) biological control of weeds has had a long history and has been one of the main strategies for the management of invasive alien plants worldwide, apart from Europe, especially using coevolved arthropod natural enemies (McFadyen, 1998; Julien and Griffiths, 1998). In contrast, the use of coevolved pathogens is still relatively new although there have been notable successes (Evans, 2002). The high success rate with arthropod agents has also been extremely cost effective, with an impres-

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sive safety record considering that over 600 agents have been released thus far (Marohasy, 1996). However, as McClayand Balciunas (2005) point out, it is a high-stakes game to achieve successful control of an invasive weed without collateral damage to non-target plants or other, more cryptic negative environmental impacts.

Paradoxically, if all of these issues were addressed, no classical biological control projects would ever get off the ground because of the prohibitive costs involved in undertaking such an in-depth ecological impact assessment. Indeed, as with any biological system, predictive models are just that—informed guesswork. There must be transparent risk assessment and on the question of risk to non-target plants the centrifugal phylogenetic protocol originally proposed by Wapshere (1974) has proven to be extremely robust (Marohassy, 1996; Evans, 2000). However, as has been stressed recently, classical biological control could be viewed as the introduction of yet another alien species: '…an issue of growing public concern' (Miller and Aplet, 2005). Although these misgivings were aimed specifically at the situation in the United States, where legal issues concerning hidden regulations have been identified, decision-makers in Europe would have taken note. In a 'blame culture', perhaps this will color decisions about future project proposals, not to say gaining permission to release classical agents in Europe.

As highlighted by Schroeder and Müller-Schärer (1995), although Europe has been a source of classical biological control agents for use in other continents for over 30 years, only two biological control programmes had been implemented within Europe (Sheppard *et al.*, 2006). One of these programmes was targeted at invasive bracken (*Pteridium aquilinum* (L.) Kuhn) in the UK but it foundered on legal, political and environmental issues (Lawton, 1988). A potential biological control agent, a leaf-feeding lepidopteran from South Africa, expired in quarantine after lengthy wranglings over additional testing and release protocols. One somewhat bizarre recommendation involved caged releases on bracken infestations in the Isle of Man: The suggested cage size was such that it was deemed both physically and economically impractical (CIBC, 1988, unpublished). Based on weed biological control success stories elsewhere, principally in Australia and South Africa, as well as the changing public opinion concerning biodiversity and conservation, Schroeder and Müller-Schärer (1995) concluded that Europe was ready for classical biological control. Amongst the weeds they identified as potential targets were giant hogweed, Japanese knotweed, and Himalayan balsam. Earlier, Evans and Ellison (1990) also considered these same invasive neophytes to be ideal candidates for this management strategy in the UK because of their increasing environmental or amenity importance. Since this time, biological control programmes have been initiated against these three weeds and, therefore, they make ideal examples to illustrate the opportunities and the constraints for the classical biological control of weeds in Europe.

**Opportunities and constraints: examples from projects:** *Giant hogweed*. *Heracleum mantegazzianum* Sommier and Levier is both an environmental and a health-threat in western Europe and an EU-funded, multidisciplinary project on its ecology and management was initiated in 2002. The outputs of this three-year programme have recently been published (Pysek *et al.*, 2007). Unfortunately the biological control component failed to positively identify any potential biological control agents following surveys for natural enemies in the plant's centre of diversity, the Caucasus Mountains. A damaging leaf-spot pathogen, *Phloeospora heraclei* (Lib.) Petr. showed exceptional promise but further evaluation was suspended when it was found to attack several related crop species, including parsnip, in lab-based screening trials. Nevertheless, questions remain unanswered concerning its true (field) host range because this pathogen has never been reported as a disease problem in cultivated parsnip, either in the UK or mainland Europe. Neither has it been found on *H. mantegazzianum* in its invasive range, despite records of its occurrence on indigenous *Heracleum* spp. (Seier and Evans, 2007). It would appear, therefore, that pathotypes or *formae speciales* specific to *H. mantegazzianum* occur. However, because of scientific and legislative uncertainties, as well as negative public perception or wariness about pathogens (pathophobia), it was decided that this would not be a good model system to launch the classical biological control concept in Europe although it was also concluded that: 'This does not negate the potential for weed biological control in Europe' (Cock and Seier, 2007). Nonetheless, how the decision makers and stakeholders in the EU have viewed this 'failure to deliver' remains unclear but it is tempting to speculate that it will only provide additional ammunition for critics of classical biological control.

*Japanese knotweed*. This project, funded by a consortium of UK environmental agencies and local stakeholders, is still ongoing and has now reached the critical phase of submitting the research data on two Japanese natural enemies of *Fallopia japonica* (Houtt.) R. Decr. for permission to release in the UK, following a four-year assessment. One of these is a hemibiotrophic fungus (*Mycosphaerella* sp.), ubiquitous and damaging in Japan and specific to the target weed (Kurose *et al.*, 2006). The legislative hurdles still to clear are discussed comprehensively elsewhere in this Proceedings (see Ehlers, R.), as well as in the literature (Sheppard *et al.*, 2006), and at first sight appear to be daunting. However, as well as a test case, this project could serve as the flagship to launch classical biological control into European waters. It is now very much a high-profile weed in the UK, receiving considerable publicity because of its diverse impacts—in natural ecosystems, as a riparian invader; in business/industrial situations, where it dramatically increases the costs of

construction work; and last but not least, at the urban or 'grassroots' level, contaminating gardens and devaluing property. It has also been in the public domain since London was chosen as the venue for the 2012 Olympics, as the Lea Valley olympic site is infested with both Japanese knotweed and giant hogweed. Initial estimates have put the costs of clearing knotweed from an area smaller than an Olympic swimming pool at around £50,000 or US$100,000 (*The Guardian*, 13 Sept. 2005). More recent reports on treating the whole site to remove *F. japonica* declared that it would add nearly £70 million to the already burgeoning and highly controversial budget (*Sunday Times*, March 2007). Such is its infamy that *F. japonica* has now entered the national psyche and vernacular, with one newspaper choosing to describe the 'success' of a certain supermarket chain as '. . . spreading through the UK like knotweed' (*The Guardian*, 19 April 2007). In fact, an official study on the costs involved in the conventional control of this weed in the UK has arrived at the 'worryingly precise' figure of £1.56 billion (DEFRA, 2003).

*Himalayan balsam.* This is the new project on the block, receiving seed money from several UK agencies to undertake a preliminary survey in parts of its native range in the lower Himalayan region of Pakistan. The results have proven to be extremely encouraging with several fungal and insect natural enemies showing good potential. However, there is still considerable onus on the scientists to raise the stakes with the donors in order to achieve the funding necessary to implement a viable biological control programme against this increasingly problematic and invasive riparian weed in the UK.

## Inundative

**Stability of research:** Besides the 'scientific' constraints that limit weed biological control in Europe, the main problems are the low number of stable or established groups working on inundative weed biocontrol strategies in Europe and the low number of projects that are or have been internationally funded on this topic. The European Framework Programmes, which represent the main source of cooperative research funds, have supported only a limited number of projects specifically dealing with biological control of weeds (http://cordis.europa.eu/en/home.html). In particular, within the 5th Framework only one project was funded. This has been mirrored in the recently completed 6th Framework, where only one project devoted to enhancing and exploiting biocontrol agents, including weed pathogens, was funded. Within the COST programme (European Co-operation in the Field of Scientific and Technical Research), one of the longest-running instruments supporting cooperation among scientists and researchers across Europe, few projects received funding, including 'Biological control of weeds in Europe' (COST 816, 1994-1999) and 'Parasitic plant management in sustainable agriculture' (COST 849, 2001-2006), which included a biological control component (http://www.cost.esf.org/).

**Favorable and unfavourable legislation:** Thanks to the enforcement of the pertinent EU directives (e.g. 91/414, 2002/2076), many dangerous pesticides have been banned in the EU countries in recent years or are scheduled to be banned within 2007. Since 2001, the use of 127 active ingredients has already been prohibited, and this list should lengthen by 14 more by the end of 2007. Among the 191 compounds recognized as herbicidal active ingredients, 71 of them were already excluded by the so-called Annex 1, which is the list of the permitted herbicides, and many others are under evaluation for their prospective exclusion (data gathered from http://fitorev.imagelinenetwork.com). This should give a renewed impetus to research on mycoherbicides and could revitalize public interest in weed biological control.

However, the Commission Directive 2001/36, which amended the Council Directive 91/414/EEC specifically for biopesticides, is very restrictive with regard to the procedures of risk assessment, registration and use of microbial plant-protection products. This is a further and potent reason why no microbial products are currently included as bioherbicides in the register kept by the Directorate of the Consumer Health Protection.

**Choice of suitable targets and efficacious agents:** The choice of an appropriate target weed is of utmost importance for the success of any biocontrol programme. Weeds that cannot be controlled by traditional methods or by chemicals due to resistance and/or environmental factors should be the preferred targets. The lack of alternative control strategies should increase the acceptability of a biological control agent, even if the price is higher due to a more sophisticated method of application. For example, parasitic plants such as *Orobanche* spp. could represent ideal targets. These weeds represent an unsolved and increasing problem in many countries of the Central European and Mediterranean regions due to their complex life cycle and lack of available control methods, especially in light of the ban on methyl bromide and other dangerous soil fumigants. In addition, perennial weeds, such as *Cirsium arvense* (L.) Scop. or *Sonchus arvensis* L., which occur throughout Europe and whose control appears particularly problematic because of their vegetative spread by subterranean organs, could be appropriate targets at the European level. Even plants spreading in anthropic environments or causing health problems (e.g. the allergenic plant *Ambrosia artemisiifolia* L.) could be perfect targets for biological control.

The search for suitable agents has not been pursued to the extent that it has been in other parts of the world, due mainly to funding restrictions (see above). The Mediterranean basin or the Caucasian region, for example, has more often represented sources of agents for classical weed biocontrol programmes overseas rather

than has been considered a source of potential mycoherbicides for European programmes.

**Improving production:** Mycoherbicides can only compete successfully with chemical herbicides if the products are as effective as the chemicals they seek to replace and if they are not significantly more expensive. Apart from the efficacy of the strain of the microorganism used, this is mainly dependent on how it is produced and formulated. The production technology used must ensure the highest possible yield of live propagules and the formulation must be such that an application of the propagules to the soil or to the plant is as easy or nearly as easy as the application of a chemical pesticide. For the development of biological control agents it is usually stated that the formulation must improve or at least assist the effectiveness of the microorganism and must ensure a shelf life of the product of at least one year, preferably two or more years. Most probably the main element is cost-effectiveness in order to make the new product competitive with current technologies. With modern inventory control, shelf life is less important, and if a product is truly efficacious but requires special application techniques or equipment, farmers will make the necessary investments just as they have for other specialized machinery now regularly used in modern agriculture.

Suitable culture media for the production of fungal propagules should be selected using an appropriate fermentation technology, followed by: evaluation of the most suitable growth conditions; selection of the best technology to separate the propagules from the fermentation product; evaluation of the most suitable methods and conditions for the formulation of the propagules produced; and assessment of the shelf life of the formulated products. The use of biomasses obtained as residues of industrial food processing or agricultural practices could reduce the cost of production. Molasses, exhausted olive cakes, residues from breweries and waste from tinned fruit industries are a few examples of such by-products that still contain nutrients and could be exploited to grow microbes. Often their disposal presents both an economic and environmental problem. Therefore, they could be ideal and inexpensive media for the production of many microbial biocontrol agents.

**Application systems:** One of the main problems in using microbial biocontrol agents to control weeds is to find suitable methods of application that allow the uniform distribution of the agent at the desired site, without waste or excessive consumption of the mycoherbicide that would increase the cost of treatment and the risks of non-target effects. The uniform and precise application of microbial particles close to the target weed and to the crop to be protected can increase the success of a biological control treatment, and the use of systems or technologies which are usually available in agriculture could influence the acceptability of biocontrol agents by farmers and enlarge the market. For example, the use of drip irrigation systems for the application of suspensions containing conidia of potential mycoherbicides has recently been suggested (Boari *et al.*, 2007). An advantage of using propagules of soil-borne pathogens that normally infect at or below the soil surface is that the propagules may be more protected from environmental factors such as wind and UV radiation, which can negatively affect conidial viability and uniformity of distribution. Applying fungal inoculum by drip irrigation does not require growers to invest in new equipment for application since this strategy is already quite widely used in agriculture to supply water, nutrients or chemicals, especially in vegetable crops where perennial and parasitic weeds often represent difficult problems. A further advantage could be the limiting of the applied doses to the crop root zone and not to the whole field, and therefore a reduction in the cost of treatment.

Several potential mycoherbicides could be applied at the soil level (Charudattan, 2001), as could microbial antagonists (Whipps and Lumsden, 2001) and biopesticides (Copping, 1998), during transplanting or through soil-drenching or root-dip, although these techniques of application can be expensive. As the fungal community already in the soil can affect the persistence of microbial treatments, longer watering intervals involving multiple treatments with lower concentrations of spores could be considered. This would result in a better distribution of the microbes in the soil in terms of volume of protected soil and amount of inoculum and reduce the risk of clogging the dripper.

Leaf-applied mycoherbicides could take advantage of sophisticated technologies, such as the use of advanced optics and computer assistance to sense if a weed is present. In this way, a precise amount of mycoherbicide could be applied only to the weeds and not wasted on bare ground. Such systems could be used where weeds occur intermittently, optimizing the consumption of spray suspension, and thus reducing the treatment costs.

**Potential for genetic enhancement of pathogen biological control agents:** Several transformation-based techniques allow reproducible genetic modifications in fungi. It should be possible to knock out genes in a biological control agent, as well as to transfer specific genes into it, and then determine effects on pathogenicity/virulence. Gressel *et al.* (2007) have recently inserted into some promising biological control agents genes considered ‘soft’, such as those encoding carbohydrases, auxin and oxalate, or ‘hard’ such as those encoding phytotoxins.

**Physiological enhancement of biological control activity:** Different approaches are being used to increase the efficacy of biological control agents without using genetic or transgenic manipulation. The transgenically enhanced hypervirulence of a biological control agent has the advantage of constitutiveness; it is already present, and there is no need for additives. Conversely, if the same effect can be achieved physiologically with

an additive, then there is the advantage that the organism is no different from the wild type after the additive has gone. For example, an organism could be engineered to overproduce oxalate (Gressel, 2002) in order to overcome calcium-dependent weed defences, or the biological control organism could be provided with exogenous oxalate to achieve similar hypervirulence (Gressel *et al.*, 2002); yet, the organism lacks hypervirulence when the oxalate is not present.

Another possibility is the use of natural mutants, such as those that are able to overproduce and excrete amino acids that are inhibitory to the target plant, resulting in enhanced virulence and improved efficacy of the bioherbicide (Thompson *et al.*, 2007).

**Environmental impact:** One of the main constraints to the release of microbes in the environment for weed biological control is the lack of knowledge about issues such as the fate of the strains after their release into the environment, their stability and the risk of dispersal. The environmental impact of a variety of biological control agents can be assessed by tracking their movement, assaying non-target \effects and any changes in host range (especially after genetic or physiological modifications), together with determining long-term environmental persistence. The introduction of biological control agents into soil may pose a risk of unforeseen or detrimental activities on the soil microbial population. The EU Directive 2001/36 states clearly that side effects on non-target soil microorganisms should be addressed, but there are no validated methods available. Until recently, techniques for monitoring direct effects on microorganisms have been restricted to *in vitro* culture-based methods that ignored 90% or more of the microbial population that could not grow on culture media in the laboratory. The development of DNA-fingerprinting techniques makes it possible to compare the genomes of all strains and to use molecular markers to recognise strains of biological control agents after their release into the environment. The study of the microbial community composition can be based on the direct extraction of DNA from soils or other complex matrices. Practically, techniques such as terminal restriction fragment length polymorphism analysis (T-RFLP), ribosomal intergenic spacer analysis (RISA) and AFLP are relatively rapid, economically feasible, and within the technological capabilities of most microbiological research laboratories. The genetic diversity within species can also be determined by using DNA molecular analyses such as sequencing of nuclear ribosomal DNA, or the beta-tubulin, calmodulin, or elongation factor genes. In addition, real-time PCR techniques can be set up for the qualitative/quantitative detection of DNA from biological control agents (Anderson and Cairney, 2004).

**Integration between diverse biological control agents, bioactive fungal metabolites, herbicides, or other chemicals:** An important factor that can reduce the efficacy of a potential mycoherbicide is the ability of the target weed to resist invasion and colonization by the biological control agent. Several attempts have been made to combine mycoherbicides with bioactive metabolites, in order to enhance agent efficacy. Such combinations can suppress or weaken plant defence mechanisms by blocking the synthesis of secondary plant metabolites or breaking down physical barriers to pathogen attack, resulting in increased biological control (Duke *et al.*, 2007, and references therein cited).

The effect of herbicides on plant disease is an important but generally overlooked aspect of integrated weed management. Nevertheless, understanding herbicide/plant pathogen interactions can be critical in designing effective and efficient integrated weed management programmes.

Synthetic herbicides have the potential to influence plant disease by several mechanisms. It is not unusual for low rates of herbicides to stimulate *in vitro* pathogen growth or sporulation (Wyss *et al.*, 2004; Yandoc *et al.*, 2006). On the other hand, herbicides such as glyphosate can also be very effective at lowering plant resistance to pathogens and acting as a synergist for microbial weed biological control products (Duke et al, 2007). In such strategies, dose rates are likely to be highly important to both the direct and indirect effects of herbicides on plant disease.

Enhanced bioherbicidal efficacy of *Exserohilum monoceras* (Drechs.) K. J. Leonard and E. G. Suggs on *Echinochloa crus-galli* (L.) Beauv., a weed in paddy rice (*Oryza sativa* L.), was obtained when the fungus was applied with δ-aminolevulinic acid, a precursor of tetrapyroles—compounds which are involved in the bleaching and killing of plant tissue (Hirase *et al.*, 2006). A system to integrate low doses of glyphosate with a foliar desiccant (ammonium sulphate) and the biological control agent *Alternaria destruens* (Smolder) to control *Cuscuta pentagona* Engelm., has also been reported (Cook *et al.*, 2005).

The efficacy of a weak biological control agent, *Colletotrichum coccodes* (Wallr.) S. Hughes, on velvetleaf (*Abutilon theophrasti* Medik.) was improved by applying calcium chelators that repressed host-plant defences by reducing callose formation (Gressel *et al.*, 2002). Also phytotoxic metabolites produced by plant pathogens can weaken defence mechanisms of plants, rendering them more susceptible to pathogen attack. Thus, the application of toxins jointly with the pathogens could strongly enhance their bioherbicidal properties (Vurro, 2007).

A mixture of three host-specific pathogens: *Alternaria cassiae* (specific to sicklepod), *Phomopsis amaranthicola* Rosskopf, Charudattan, Shabana & Benny (specific to pigweeds), and *Colletotrichum dematium* f. sp. *crotalariae* (specific to showy crotalaria), proved to be very efficacious in the simultaneous control of the three weeds (Chandramohan and Charudattan, 2003). Good control of seven weedy grass species has also been obtained using a

suspension containing conidia of three host-specific pathogens: *Drechslera gigantea* (Heald and Wolf) Ito, *Exserohilum rostratum* (Drechsler) Leonard and Suggs, and *Exserohilum longirostratum* Subram. (Chandramohan *et al.*, 2002).

## Discussion

Perhaps we can borrow the use of the 'Anna Karenina Principle' from McClay and Balciunas (2005), who first applied it to biological control [the list of borrowers, of course, goes back to Tolstoy (1877)] in order to compare the constraints and opportunities for classical vs inundative biological control. For the inundative approach, these are essentially similar for every weed target in every country, region or continent: 'happy families are all alike', and the issues involved have been addressed here. However, for the classical approach, especially against invasive environmental weeds, the factors involved are extremely, and often uniquely, complex and therefore must be dealt with on a case-by-case basis: 'every unhappy family is unhappy in its own way'.

In the European context, many of these issues have already been highlighted and reviewed comprehensively by Sheppard *et al.* (2005). Suffice it to say that no biological control agents have been released thus far, and the few that are in the pipeline face an uphill struggle and uncertain future for acceptance, despite the fact that: 'Classical biological control remains the only tool available for permanent ecological and economic management of invasive alien species …' (Sheppard *et al.*, 2005). This approach has even received the seal of approval from the Convention on Biological Diversity (CBD), the European and Mediterranean Plant Protection Organisation (EPPO) and the European Strategy on Invasive Alien Species (ESIAS). However, is this the kiss of death, as the bureaucratic red tape kicks in? As previously mentioned, there are now so many more environmental concerns to address, compared to earlier times, that the costs of implementing all of them would put any biological control project out of the financial reach of traditional donors. Certainly, we have moved on from the 'hunter-gatherer, quick-release, let's-try-this-one' approach to the position where it is essential to abide by the CBD and to undertake scientifically driven risk assessments. These are in place but still subject to the whims and interpretations of individual countries and international organisations, as well as the critics of biological control. In the present climate, it would still take only one mistake, or unexpected non-target issue, to seriously undermine the solid scientific foundations on which classical biological control is based.

In the case of the CBD, this has recently created additional barriers hampering free exchange of germplasm between countries. For example, permission to release an Argentinian strain of the rust, *Puccinia spegazzinii* DeToni for use against the highly invasive mile-a-minute weed (*Mikania micrantha* Kunth) in China has been blocked, seemingly permanently, by Misiones Department, which has a separate CBD policy from that of Argentina. Thus, biological control scientists are now expected to negotiate delicate political issues in order to implement classical projects. In addition, there are constant attacks on or criticisms of the safety-testing procedures employed to screen classical weed biological control agents, despite an impeccable track record (McFadyen, 1998), as well as new concerns about the indirect impacts of even host-specific agents on non-target species (Pearson and Callaway, 2003; Louda *et al.*, 2003). These concerns provide further ammunition for biological control sceptics to shoot down any proposals, which are based solely on the classical biological control approach, before they have gotten off the ground. Moreover, this relates only to relatively 'inoffensive' insect agents! What hope is there for pathogens? It could be argued that if all the environmental concerns and risks involved in undertaking a motorized shopping trip were analysed as critically, supermarkets would go out of business (even the ones spreading like knotweed)!

As flagged by Thomas *et al.* (2004), such concerns serve to highlight '... the need for proper ecological and socioeconomic evaluation of pests before control to determine probable costs and benefits'. The financial stakes are raised yet again, as well as time frames, before biological control can even be considered as a management option, putting such proposals on a different donor level. The giant hogweed project, for example, was large by EU standards because of its multidisciplinary approach and composition. Even so, fundamental questions relating to the safety of several potential biological control agents still remained unanswered because of insufficient time and funding. Thus, we are left with the possibility that future classical weed biological control projects for Europe need to be multidisciplinary and last a minimum of 5–10 years to achieve all the goals now expected, once the target posts have been moved. Such multi-million euro proposals must thus be the norm if proposals are to be approved and project aims realized. It is no coincidence, perhaps, that the multinational, multi-organisational, multidisciplinary biological control project against migratory locusts did achieve its objectives, but only after a massive injection of funds from a consortium of international donors for over more than a decade.

This leads finally to the perennial question: biological control—risky or necessary? (Thomas and Willis, 1998). Critical decisions need to be taken in Europe regarding the long-term management of invasive weeds, especially those with serious environmental impacts, but perhaps it is better not to fiddle around too much while the aliens continue their destruction of fragile ecosystems.

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# Could *Fallopia japonica* be the first target for classical weed biocontrol in Europe?

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*Summary*

Japanese knotweed, *Fallopia japonica* (Houtt.) Ronse Decr., (Polygonaceae), is a serious environmental and economic weed in its adventive range of Europe and much of North America. Such is the scale of the problem in the UK that a pioneering biocontrol programme began in 2003 which would possibly make it the first target of a full classical biological control of weeds programme in Europe. This paper summarizes the current status of the plant, reviews the literature associated with its natural enemies and reports the progress with the programme for UK sponsors, as well as referring to North American interests. We conclude that, should appropriate permissions be made available, the prospects for biological control of this high profile weed, using arthropod and fungal agents, are good.

***Keywords***: Japanese knotweed, classical biological control, *Fallopia japonica.*

## Introduction

In December 2003, the European Strategy on Invasive Alien Species (ESIAS) came into being (Genovesi and Shine, 2004), supporting the Convention on Biological Diversity (CBD), calling for a regional approach to the invasive alien species problem, and highlighting the need for cost/benefit analyses of long-term control measures. Any country intending to control those invasive alien species that threaten ecosystems, habitats or species are encouraged by the Convention to consider classical biological control for environmental weeds.

There have been over a thousand releases of biological control agents against weeds worldwide. Despite European countries being the source for 381 releases of classical biological control agents for alien plants around the world (Julien and Griffiths, 1998), no full classical weed biocontrol programme has yet been carried out for the benefit of an EU country. The reasons for this are manifold and a source of frustration for many weed biocontrol experts working in Europe, particularly in light of the long list of potential targets (Shaw, 2003; Sheppard *et al.*, 2006). *Fallopia japonica* (Houtt.) Ronse Decr., Japanese knotweed (Polygonaceae), is one such target whose profile is so high that many of the usual hurdles have been easier to overcome than for previous potential targets.

### Nomenclature

Japanese knotweed was independently classified as *Reynoutria japonica* by Houttuyn in 1777 and as *Polygonum cuspidatum* by Siebold in 1846. It was not until the early part of the 20th century that these were discovered to be the same plant (Bailey, 1990). Generally referred to as *Polygonum cuspidatum* by Japanese and American authors, recent evidence vindicates Meissner's 1856 classification as *Fallopia japonica* var. *japonica* (Bailey, 1990). The closely related giant knotweed, *Fallopia sachalinensis* (F. Schmidt ex Maxim.) Ronse Decraene can hybridise with *F. japonica* to form *Fallopia* x *bohemica* (Chrtek and Chrtková) J. Bailey, first described in 1983, and is rapidly proving to be more difficult to manage than either of its parents (Bímová *et al.*, 2001; Mandák *et al.*, 2004).

Common names include Japanese/Mexican bamboo, pea-shooter plant, Sally/donkey/gypsy/wild rhubarb, Hancock's curse, Japanese fleece-flower and horse buckwheat. The Cornish name, 'Ladir Tir', is a rare example of the democratic addition to the lexicon since the Cornwall Knotweed Forum, voted for this translation of their preferred English descriptor, 'land thief'. Itadori, the Japanese name for the plant, translates as

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'take away pain', presumably reflecting its medicinal properties. The subject of this paper will be referred to as Japanese knotweed.

## Reproduction

In its native range, the plant is functionally dioecious but in its introduced range it has spread solely by vegetative means from a very small number of initial introductions. Consequently, much of the invasive knotweed in the world may be clonal, as is the case in the UK (Hollingsworth and Bailey, 2000). However, recent research in the USA has shown that wild *F. japonica* can produce large quantities of viable seed and seedlings have been found in the field (Forman and Kesseli, 2003).

## Morphology

Detailed descriptions of Japanese knotweed's morphology are available (Beerling *et al.*, 1994; Lousely and Kent, 1981). It is a vigorous, herbaceous perennial, with annual, glabrous, tubular stems which ascend from an often extensive rhizome system, to reach heights of over 3 m in 3 months (Beerling *et al.*, 1994).

## Spread

The history of alien *Polygonum* and *Reynoutria* species in the UK has been well reported (Bailey and Conolly, 2000; Bailey, 2005; Conolly, 1977). The most likely date of introduction of Japanese knotweed to Europe is 1849, received at the nursery of Philipp von Siebold in the Netherlands. This was also the first year that the *japonica* variety was made available to the public as a much-prized ornamental. In the UK, the plant had become naturalized by the late 1880s, having been first recorded in the wild in Maesteg, South Wales, in 1886 (Conolly, 1977). Its status as a weed was soon recognized, and today it is one of only two terrestrial plants which are 'illegal to cause to grow in the wild' under the UK 1981 Wildlife and Countryside Act, as well as being classed as a 'controlled waste', meaning that a licence is required for its disposal.

## Damage

The costs of Japanese knotweed can be considered as both economic and environmental. To control Japanese knotweed on a national scale in the UK would cost an estimated £1.56 billion, as noted by a review team reporting to the UK Department of Environment, Food and Rural Affairs in its recent non-native species policy review (Defra UK, 2003). An accepted estimate of control costs is £10,000 per hectare for a three-year spraying regime with two sprays per year, although this is probably an underestimate if revegetation costs are taken into account. With fragments as small as 0.6g capable of generating new plants, the presence of Japanese knotweed can add around 10% to the costs of a development project, especially if soil is considered contaminated and subject to removal fees. A worst-case scenario could see a 1m$^2$ patch costing up to £46,000 to eradicate (M. Wade, 2006, personal communication). Its reputation as a 'concrete-cracking super-weed' is justified; seven designs of reinforced channel revetment blocks were specifically tested against penetration and displacement by Japanese knotweed (Beerling, 1991), and all seven failed. In East London, work has begun to clear four hectares of knotweed infesting the 2012 Olympics site, an activity which is gleefully reported by the press to have added £65 million to the expanding development budget.

Though harder to quantify, the impact the weed has on ecosystem function and biodiversity are considerable. Its early emergence and great height combine to shade out other vegetation and prohibit regeneration of other species (Sukopp and Sukopp, 1988). Dead knotweed stems can persist for two to three years producing large quantities of debris and slowly decomposing litter, which also leads to low floristic diversity (Child and Wade, 2000). Observations on knotweed in the UK revealed that invertebrate species' richness was lower on *F. japonica* than on sympatrically occurring native plant species (Beerling and Dawah, 1993). More recent work in Switzerland, Germany and France, comparing the diversity of plants and invertebrates in invaded and non-invaded habitats, showed a reduced diversity on both taxa, as well as a halving of invertebrate biomass under knotweed (E. Gerber, unpublished data). Impacts on fish and other vertebrates further up the food chain are likely and knotweed-invaded sites appear to be less suitable habitats for foraging frogs (Maerz *et al.*, 2005).

Dense knotweed stands can also exacerbate flooding, damage riverbank protection works and impede flow, whilst dead stems can cause blockages downstream when swept away. Knotweed's influence on riparian systems is particularly pertinent in the light of the EU Water Framework Directive, which demands that member nation's waterways achieve 'good status' by 2015.

## Current control measures

The effectiveness of control and eradication interventions has recently been thoroughly reviewed by Kabat *et al.* (2006), who included 65 articles in their meta-analyses. Six control interventions were considered, none of which could eradicate Japanese knotweed or its hybrid in the short term. Cutting treatments alone were not found to result in significant decreases of knotweed abundance. However, they found that statistically significant reductions in abundance could be achieved through limited application of: a) glyphosate,

imazapyr, or imazapyr plus glyphosate, b) cutting followed by filling stems with glyphosate, or c) cutting followed by spraying with glyphosate. These authors were unable to conclude any clear long-term efficacy.

As a general rule of thumb, based on discussions with numerous experts in the UK and the United States, a late-season application of glyphosate, when the plant is at maximum height, is the most cost-effective control measure.

# Literature and field observations

## Methods and materials

The phytophagous arthropods and fungi recorded from Japanese knotweed were collated from the printed and electronic literature in both the English and Japanese language and, for arthropods, their feeding habits were categorized. Surveys were carried out across the growing season of the plant each year from 2003 to 2006. Early surveys covered the complete range of the plant from Northern Honshu to Southern Kyushu islands, whilst later collections focussed on Kyushu Island. The focus on Kyushu Island was a consequence of molecular studies carried out at Leicester University, that showed the closest match to the UK clone was to be found in the Nagasaki Prefecture of Kyushu. At each site, knotweed plants were first visually assessed for natural enemies, their activity and/or damage inflicted, before using a beating tray to collect any natural enemies that had been missed. A subset of the plants had their stems and rhizomes split open to reveal any endophagous species. Simultaneous assessments were carried out on other members of the Polygonaceae family growing in the vicinity to provide data on field host range.

## Results

The literature review of natural enemies revealed 186 arthropod species and over 30 fungal plant pathogens to be associated with *F. japonica* in Japan. This is in stark contrast with the situation in the UK where only 14 arthropods and no fungal plant pathogens have been recorded on the plant (Figure 1). In Japan, leaf feeders and sap suckers together made up over 87% of the arthropod species recorded (Figure 2). The dearth of rhizome feeders was notable and this surprising observation was supported by subsequent field surveys, which revealed this large resource to be almost solely exploited by the polyphagous hepialid moth *Endoclyta excrescens* (Butler).

Surveys revealed that knotweed was subject to significant—and in many cases, severe—natural enemy damage. In undisturbed areas, this led to it being outcompeted by the many large forbs characteristic of the Japanese flora. Observations on sympatric Polygonaceae revealed that a handful of these natural enemies had a very narrow host range. It should be noted that at sites where the natural enemy cycles had been disrupted by cutting, Japanese knotweed revealed its potential as a dominant species.

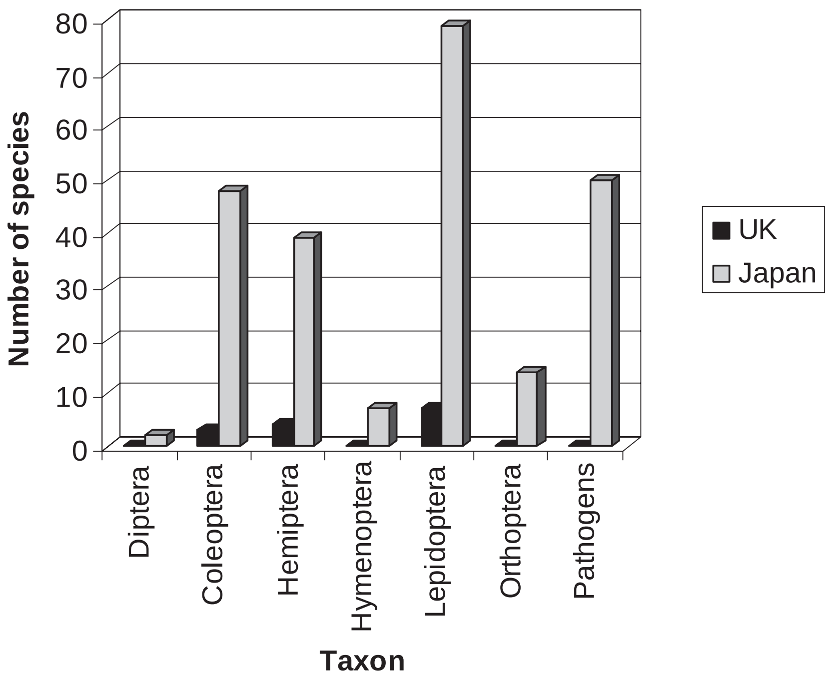


**Figure 1.** A comparison of phytophagous natural enemies recorded on *Fallopia japonica* in Japan and the UK by order (fungal pathogens are presented collectively).

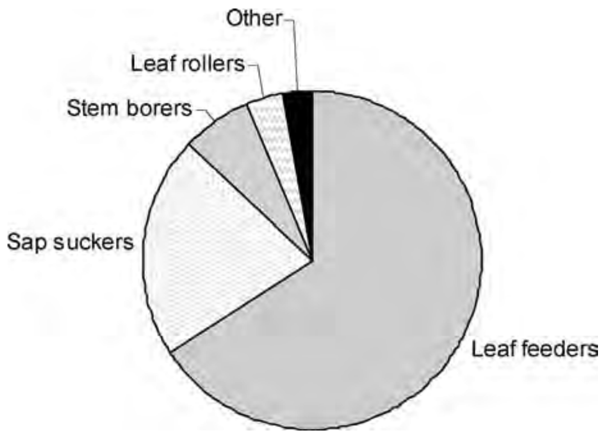


**Figure 2.** Arthropod natural enemies found on *Fallopia japonica* in Japan, grouped by feeding habit.

# Potential biological control agents

The status of the more interesting potential biocontrol agents is presented below including a summary of any host-range testing that has been undertaken. Despite the sound arguments for shorter and more tailored host-range test plant lists (Briese, 2005), the approach taken here was the more traditional one including apparently irrelevant species. This decision was taken because any consideration of the use of a classical biological control agent in the UK would be novel and the authorities are likely to be most interested in economic and crop plants. The list includes 74 species from 23 families, consisting of 33 plants native to the UK, 15 introduced species, three native to Europe, 13 ornamentals and ten economically important crop species.

## Arthropods

***Ostrinia ovalipennis* Ohno (Lepidoptera: Crambidae)** is a recently identified (Ohno *et al.*, 2003; Ohno, 2003) close relative of *O. latipennis* (Warren), a well-known and widely distributed knotweed borer feeding on other species in the field in Japan. *Ostrinia ovalipennis* appears to be univoltine and restricted to two distinct populations; one from Hokkaido Island and the other from highland areas in the Nagano Prefecture of central Japan (Ohno *et al.*, 2006). It has only been recorded from Japanese and giant knotweed. Identification and likely rearing difficulties meant that this potential agent was not prioritized for the UK but remains of interest for North America where giant knotweed is more of an issue.

***Macchiatella itadori* (Shinji) (Hemiptera: Aphididae)** is a very common aphid which causes severe damage to both *F. japonica* and *F. sachalinensis* from June to September, often in association with leafspot and various ant species that tend it. Unfortunately, its primary winter hosts are recorded as *Rhamnus japonica* Maxim and *R. purshiana* De Candolle and, as such, it was dismissed for the UK due to the likely attack of native *Rhamnus* spp. It is also unlikely to be of interest to North America, unless the latter host has been recorded erroneously.

***Ametastegia polygoni* Takeuchi (Hymenoptera: Tenthredinidae).** This stem-mining sawfly has been collected from both Japanese and giant knotweeds in the field, although the Japanese literature only reports Japanese knotweed as a host plant. Attempts to establish a culture for host-range testing have failed so far but this sawfly has not been rejected.

***Gallerucida bifasciata* (Motchulsky) syn. *Gallerucida nigromaculata* (Baly) (Coleoptera: Chrysomelidae).** Originally two species, these have recently been synonymized to *G. bifasciata*. Some doubt remains, however, since our collections revealed considerable differences in both morphology and behaviour in populations from different regions of Japan. A more southerly population was used for preliminary larval no-choice testing and when presented with both cut plant and live plant material, larvae fed significantly within the Polygonaceae family. Subsequent field observations in Kyushu revealed larvae feeding on *Rumex acetosa* L. leaves and consequently, this beetle was not prioritised. A more northerly population has been collected and is undergoing testing in the United States.

***Lixus impressiventris* Roelofs (Coleoptera: Curculionidae)** is a very common stem-boring weevil which has only ever been collected from Japanese and giant knotweeds in the field even when suitably sized stems of *Rumex* spp. were present, at a site where almost every internode section of the knotweed stems contained a *Lixus* larva. Nonetheless, adult no-choice and choice tests showed a physiological host range that included many members of the Polygonaceae. Despite a Japanese paper reporting the weevil as a pest of a

very minor crop, *Polygonum tinctoria* Lour. (Sekiguchi and Wakiya, 1988), every attempt to rear the weevil on this plant has failed. No-choice oviposition and development tests showed that one native UK plant, *Polygonum hydropiper* (L.), was able to support development of the weevil, albeit producing significantly smaller adults in the process. The possibility of adults feeding on non-targets and the risk of development on a native plant species have meant that this weevil is no longer a prioritized agent for the UK. Further studies, perhaps in the native range, may well prove this weevil to be highly specific.

***Aphalara itadori* Shinji (Hemiptera: Psyllidae)** is found from southern Kyushu to as far north as Nagano Prefecture on Honshu Island and was observed feeding on Japanese knotweed from sea level to 2150 m a.s.l. Adults were collected from late April to mid-August and although widespread, were rarely present in high numbers. One unidentified eulophid parasitoid has been reared out from a late nymph. Adults lay eggs on the leaves or under the papery sheath surrounding the petiole and once hatched, the nymphs pass through five instars feeding on the phloem of the plant. In the laboratory, at 22$^{o}$C, the mean development time was 32.9 days (±0.8= SE, n=21) and reproductive females laid a mean of 637 eggs each (±121= SE, n=11). Impact studies are ongoing but early signs indicate that the presence of feeding nymphs restricts plant height and increases leaf production.

Host-range tests have focussed on multiple-choice oviposition studies since the nymphs are not very mobile and adult feeding was hard to observe and quantify. Host-absent multiple-choice tests were used to test the validity of host-present tests and no significant difference was found when very closely related plants were used. Over a 20-month period, the location and fate of just under 125,000 eggs have been recorded during tests on 83 test plant species. So far, only 700 eggs (0.6%) have been laid on non-knotweed or knotweed hybrid hosts and not one of these has developed through to adult. Although more replication is required on some non-target species, these results are extremely encouraging. The question of what happens when above-ground knotweed dies off at the first frost remains. Adult *Aphalara* are presumed to shelter in the bark of trees such as *Cryptomeria* spp. (N. Takahashi, 2007, personal communication). This is currently being investigated.

## Pathogens

***Puccinia polygoni-amphibii* var. *tovariae* Arthur (Basidiomycota: Pucciniaceae).** Several strains of this rust have been found in the field on *F. japonica* in Honshu and Kyushu Islands, from sea level to 1550 m a.s.l. Collected all year round, either as cinnamon brown-coloured uredinia or as the over-wintering, brownish-black, telial stage, it was also recorded on *F. sachalinensis*, *F. japonica* var. *compacta* and the somewhat hairy-leaved *F. japonica* var. *uzenensis*. The uredinial spore stage was tested against more than 40 non-target plants. It showed a great deal of potential by infecting all stages of the target plant, causing severe defoliation in the lab. Unfortunately, infective symptoms and subsequent sporulation to produce viable spores were consistently recorded on the native *Rumex longifolius* DC, and *Fallopia baldschuanica* (Regel) Holub, an ornamental. Furthermore, its life cycle could not be resolved in quarantine since telial dormancy could not be broken under these artificial conditions. These facts, coupled with reports in the Japanese literature of closely related *Puccinia* varieties being heteroecious, with *Geranium* spp. as alternative hosts, meant that this damaging rust was no longer prioritized for the UK.

***Aecidium polygoni-cuspidati* Dietel (Basidiomycota: *Incertae sedis*).** This conspicuous rust was found on *F. japonica* var. *compacta* and *F. japonica* var. *japonica* in the field and has been recorded from *F. sachalinensis* in the Japanese literature. It is found from late April to September at altitudes of up to 1270 m, but occurring more frequently in the lower, warmer areas of both Honshu and Kyushu islands, commonly in humid riparian and woodland habitats. Failure to infect knotweed plants with aeciospores in the lab reinforced suspicions that this rust may in fact be heteroecious and synonymous with *Puccinia phragmitis* (Schum.) Körn., using *Phragmites communis* Trin. as its alternative host. This was confirmed in the Japanese literature (Harada, 1978) where it was identified that the rust had many specialized biologic forms or strains, one of which infected *F. japonica* and *F. sachalinensis* in its aecial stage. This agent has therefore been dismissed.

***Mycosphaerella polygoni-cuspidati* Hara (Ascomycota: Mycosphaerellaceae).** This hemibiotrophic pathogen which cycles through the sexual ascospores and causes a highly damaging and ubiquitous leafspot is found on Kyushu, Honshu and Shikoku islands, from sea level to altitudes over 900 m. Displaying a high degree of polymorphism, the lesions may appear as large, dark-tan coalescing forms, or as circular or irregular, chestnut-brown lesions, or as more discrete spotting. This, and considerable variation shown in cultured isolates, suggests that there is a range of morphotypes and/or pathotypes. The leafspot appears to be restricted to *F. japonica* var. *japonica* in the field, and coincides with knotweed stem emergence in late April through to its senescence in October/November. After considerable investigation, this slow-growing ascomycete has now been confirmed as the causal agent, following Koch's postulates. Host-range testing has been carried out, using ascospore inoculum for closely related species and mycelial inoculum for the entire host-test list since the availability of the former is limited. To date, over 50 plant species have been tested, and results confirm the extremely narrow host range shown in the field. Genetic characterization of the

various strains is being carried out to ascertain their relatedness.

## Discussion

The short answer to the question posed by the title of this paper is 'no', this will not be the first target for classical biocontrol of weeds in Europe. This is not because an eventual agent release is unlikely but rather because it would not actually be novel for Europe. Closer examination of a biological control study against creeping thistle (*Circium arvens*) in the UK in 1969 (Baker *et al.*, 1972) revealed that, although the initial releases of hundreds of adult beetles (*Haltica carduorum* Guerin) from France were made into field cages, these cages were removed later in the study. The eventual results were similar to those encountered in Canada, with no successful survival over winter (Peschken *et al.*, 1970).

Despite this, the completion of a full, official classical biological control programme for a weed in Europe is effectively a new concept and would be expected to face various challenges from the outset (Shaw, 2003; Sheppard *et al.*, 2006). A team at the University of Coimbra in Portugal is currently studying the safety and efficiency of the gall wasp, *Trichilogaster acaciaelongifoliae* Froggatt, against *Acacia longifolia* (Andr.) Willd. in quarantine. This agent was successfully released in South Africa (Julien and Griffiths, 1998). This is part of a larger project, but it could be that this excellent agent will be the first classical agent released in Europe against a weed.

Regulatory challenges are likely to be the most difficult to overcome especially when it comes to fungal agents, although proposed arthropod releases for weeds have not been welcomed as much as those for insect pests. At this stage, the psyllid *Aphalara itadori* and the leafspot *Mycosphaerella* sp. seem likely to pass the host-range testing process, but whether the prospect of an actual release into the environment becomes a reality is likely to depend on individuals within the appropriate UK government and EU department(s) taking a pragmatic approach to often inappropriate or absent legislation. If the eventual goal of release is achieved, then this programme will indeed have laid the groundwork, helped establish the rules and opened the door to classical biological control of weeds in Europe (Kurose *et al.*, 2006).

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# Biological control of *Rumex* species in Europe: opportunities and constraints

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*Summary*

The increasing problems caused by dock infestations (especially *Rumex obtusifolius* L., *R. crispus* L., and *R. longifolius* DC.) to organic agriculture in Great Britain, Norway and Switzerland are discussed. Inadequate, costly, or time-consuming non-chemical control options for *Rumex* are among the major barriers for farmers converting to organic production. Potential biological control agents for *Rumex* in Europe are discussed. We conclude that the chrysomelid beetle *Gastrophysa viridula* Degeer and the rust fungus *Uromyces rumicis* (Schum.) Wint. remain the most promising of the researched indigenous species and that *G. viridula* can be combined with other non-chemical control methods. However, there is a need for biological control agents that target dock roots; we suggest that *Pyropteron chrysidiformis* (Esper), one of several sesiid moth species present in Europe which attack dock roots, has good potential for *Rumex* spp. biological control and merits further study within Europe.

***Keywords***: dock, organic farming, *Rumex crispus, Rumex longifolius, Rumex obtusifolius.*

## Introduction

Docks, especially *Rumex obtusifolius* L. and *R. crispus* L., have been recognized as problem weeds in conventional agriculture for centuries (Foster, 1989; Zaller, 2004). These species grow rapidly, are resilient to cutting (being able to quickly regrow from their root stock, and replenish carbohydrates used in regrowing within two to three weeks), are long-lived and are able to produce up to 80,000 seeds per plant per year (Cavers and Harper, 1964). These seeds form a long-lasting soil seed bank with seeds surviving for possibly up to 80 years (Cavers and Harper, 1964). More recently, docks have been recognized as a serious problem for organic agriculture and an important limiting factor in the conversion from conventional to organic farming is thought to be the worry of many farmers over their ability to control docks without chemical herbicides. In this paper, we examine this problem as well as recent and ongoing research into it in three European countries—Great Britain, Switzerland and Norway. We discuss possible biological control methods (none of which are currently used in Europe) and our recommendations for the way ahead in Europe. Of course, this paper only gives a snapshot of the situation in three countries and there is much work also taking place in other European countries—for example, Germany (Zaller, 2004), the Czech Republic (Martinková and Honěk, 2004) and Austria (Hann and Kromp, 2003). Also, as we do not intend to review the voluminous work on non-chemical control of *Rumex* spp., readers are referred to Foster (1989), Hatcher and Melander (2003), Zaller (2004) and Bond *et al.* (2006) for this.

## The problems

### Great Britain

In January 2005, a total of 690,269 ha of agricultural land in the UK was registered as organic or in conversion to organic; just over 4% of all agricultural land (FiBL, 2006). Of this, 92% was fully organic and the retail market for organic products in the UK was worth GBP 1.213 billion in 2006. The problem caused by docks to UK organic producers became very apparent during the course of a three-year UK Government

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(DEFRA)-funded research programme into the management of weeds in organic production systems, carried out by the Henry Doubleday Research Association (HDRA) (Turner *et al.*, 2004, 2007). Of those farmers surveyed, 92% listed *Rumex* spp. as one of their main problem weed species. Farmers had a clear understanding of what encouraged docks in their systems (e.g. poaching, inappropriate or untimely cultivation) and many thought that their dock problems were historical and had persisted due to poor weed management prior to organic conversion. The general approach to control was to prevent dock seeding and to reduce their vigour by harvesting crops before docks seeded, keeping margins clean, planning control periods into rotations and the use of grazing stock. Direct action included various integrated topping and grazing strategies, with many farmers using topping machinery. Sheep were used to intensively graze young seedlings, and goats to strip mature plants. Manual removal was also used at times, and periods of summer fallow were used to cultivate the land, cutting roots below 10 cm and exposing the roots on the surface to desiccate (to prevent regrowth). Raking off these roots and burning them was effective.

## Switzerland

Switzerland was one of the pioneers in organic farming, and there were already 500–1000 such farms in the 1960s (Niggli, 2005). From the 1940s there was a steady increase in conversion to organic farming and since the 1990s this conversion has increased rapidly: in 1990 there were 803 organic farms totalling 10,000 ha; by 2005 there were 6462 of 112,000 ha comprising roughly 10% of farms and cultivated land in the country (Niggli, 2005). These farms are typically small, with an average size of 14 ha in 1998. Farmers in Switzerland have identified *R. obtusifolius* and other dock species (e.g. *R. crispus, R. alpinus* L.) as a major limitation to plant production on existing organic farms and a serious obstacle to conversion to this type of production (LBL Bericht, 2001). Organic farmers are typically prepared to put considerable time into weed control with some devoting over 1000 man-hours per year to dock control alone (Grossrieder and Keary, 2004) but this amount of effort is not feasible for all farms and is obviously limited by economics. Large-scale physical control using machines was not suitable as it causes soil disturbance and this promotes dock seedling establishment. Variation in cultural control methods, such as cutting height and frequency, grass species sown, and added nutrients have all been found to have only limited effects on dock populations. A review of these grassland experiments (Lüscher *et al.*, 2001) demonstrated that all these management options did not significantly reduce the competitive ability of established *R. obtusifolius* plants. Dock seedlings, however, have a much weaker competitive ability than most sown grasses. Consequently, all measures that increase grass sward density and prevent gaps are successful against the new establishment of dock plants, and root competition was much more important than shoot competition [this has also been shown for *R. longifolius* in Norway (Haugland, 1993)]. Such cultural control methods must be part of a holistic management strategy if the weed is to be controlled (LBL Bericht, 2001; Niggli, 2005) and should focus on preventing the establishment of new dock plants (Dierauer *et al.*, 2007). Due to the restrictions on control methods in organic farming, biological control is a logical tool to be integrated into such a strategy.

## Norway

Organic farming started in Norway in the 1930s, but there were few such farms until the 1970s. A national organic certification procedure was adopted in 1986, with 19 farms being certified originally, and since then the number of organic farms has increased steadily. In 1996 there were 946 farms of 7900 ha total (0.8% total agricultural land) (Johnsen and Mohr, 2000), while by 2006 there were 2500 farms of 38,798 ha (3.8% of agricultural land) (Debio, 2006). At 13 ha, the average organic farm size is slightly larger than that of conventional farms, and almost all are run as family farms. The current national ‘plan of action’ aims for 15% of agricultural land to be organically farmed by 2015 (www.regjeringen.no).

Over 80% of organic agricultural land in Norway is under grassland, meadows or green manure, 15% under cereals, with little organic horticulture (Debio, 2006). Thus, grassland weeds are a major problem. A report from Sweden (Andersson, 2005) states that many farmers feel powerless to control their *Rumex* problem, and some organically motivated farmers are prevented from converting to organic production because of this. This applies also to Norway. Along with *R. crispus* and *R. obtusifolius, R. longifolius* DC is also present in Norway. It is the most widespread weedy *Rumex* species (Fykse, 1986) and is one of the most troublesome dicot species in Norwegian grasslands (Haugland, 1993). *R. longifolius* can grow up to 1250 m above sea level; it develops much faster in the spring and forms twice as many shoots from root fragments but regrows slower after defoliation than *R. crispus* and *R. obtusifolius* (Fykse, 1986). A major Norwegian study has started to investigate the natural enemies and control options for *R. longifolius* and other *Rumex* spp. in Norwegian organic agriculture (Brandsæter and Haugland, 2007).

# Biological control

## Insects

Grossrieder and Keary (2004) have reviewed recent studies on potential insect biological control agents for *Rumex* spp. Much of the research reported here was

carried out on European species of potential for *Rumex* and *Emex* biocontrol in Australia, and has concentrated on agents with a southern European, or Mediterranean distribution, to match that of Australia. It is unlikely that these species will be suitable for biological control in the three central and northern European countries considered here, ruling out species such as *Lixus cribricollis* Boheman (Col., Curculionidae) and *Synansphecia doryliformis* (Ochsenheimer) (Lep., Sesiidae). Nor is it likely that introducing non-indigenous biological control agents will be feasible in Europe within the near future, due to the current regulatory climate. There is only one species in this category that might be worth investigating at present: *Gastrophysa atrocyanea* Motschulsky (Col., Chrysomelidae) from Japan, which is probably the most promising insect for classical biological control of *R. obtusifolius*.

Within Europe, we need to concentrate on those insect species that can cope with the current management regimes used against docks (e.g. cutting, ploughing, removal before flowering) and this eliminates further species. For example, several species of *Apion* weevils are found on *Rumex*. These mainly bore into the flowering stem as larvae, and adults emerge in July to August, after flowering. However, no organic farmer is likely to leave a flowering *Rumex* in their fields if they can help it, although this could be considered in uncultivated areas. Biocontrol agents should also be easy to rear or culture for potential inundative releases.

*Brachycaudus rumexicolens* (Patch) (Hom., Aphididae) is recommended for further study by Grossrieder and Keary (2004). First discovered in the US in the early 20th century, this species is confined mainly to the Polygonaceae (Scott and Yeoh, 1998), especially *Rumex* and *Emex*, although it can also attack *Lupinus albus* L. and *Triticum aestivum* L. and it may also be a virus vector. Nevertheless, the aphid was considered sufficiently safe to be used in a programme aimed at the biological control of *Emex australis* Steinh. in Australia (Scott and Yeoh, 1998). This sap-feeding insect caused widespread death and stunting of *Emex*, reducing individual achene weight by 41% (Scott and Shivas, 1998), and has a high intrinsic rate of increase [$r_m$ alatae = 0.32, apterae = 0.43 at 24°C (Scott and Yeoh, 1999)] even for an aphid. Scott and Yeoh (1999) carried out temperature studies and bionomic modelling on *B. rumexicolens* and showed that it should be able to survive in northern Europe. It has already been recorded from most of Europe, including the UK and Norway (e.g. Ossiannilsson, 1962).

*Hyperia rumicis* L., a leaf-feeding weevil (Col., Curculionidae), and *Pegomya nigritarsis* (Zetterstedt) (Dipt., Anthomyiidae), a leaf miner, can both occasionally cause extensive damage to docks (Grossrieder and Keary, 2004) but seem to have few advantages over the leaf beetle *Gastrophysa viridula* Degeer (Col., Chrysomelidae).

*Gastrophysa viridula* is the most-studied dock insect. It has up to four generations a year in Europe, overwintering as an adult in the surface layers of the soil, and passing through a generation in six weeks in favourable conditions throughout the spring to autumn. The species can show a large population increase during the year; with females able to lay over 1000 eggs each. Outbreaks of this insect have been reported, stripping *Rumex* plants of leaf material. Although Martinková and Honěk (2004) report that it will feed upon nine other plant families, it can only complete its lifecycle on *Rumex* spp. and prefers *R. obtusifolius* to other docks (Bentley and Whittaker, 1979). Dispersal of the beetle is limited; it has been rarely observed to fly and tends to occur in discrete patches. Martinková and Honěk (2004) suggest that the beetle has become more widespread in central Europe during the 20th century, with a recent expansion since 1950 with the spread of weedy docks in lowlands during the formation of large farms. *G. viridula* can cause up to 50% reduction in dry weight of *R. obtusifolius* during the first year of growth (Hatcher *et al.*, 1997), up to 80% shoot and 65% root reduction of *R. crispus* and *R. obtusifolius* first-year overwintering plants (Hatcher, 1996), and can cause up to 70% reduction of dry mass and 65% reduction in seed production in the first four years of *R. obtusifolius* growth in the field (Hatcher, unpublished data).

Several species of clearwing moth may be suitable biological control agents. While *Synasphecia doryliformis* has a Mediterranean distribution, the closely related *Pyropteron chrysidiformis* Esper (Lep., Sesiidae) is native throughout western Europe and southern England, but has not been recorded from Scandinavia (Spatenka *et al.*, 1999). As in the case of *S. doryliformis*, the species is univoltine and the larvae feed in the roots of various *Rumex* spp. (Spatenka *et al.*, 1999). *Synasphecia doryliformis*, which was mass-released into Australia in the early to mid-1990s as a biological control agent against docks (Fogliani and Strickland, 2000), reduced dock densities there by up to 90% within five years of release (Faithful, 2000). Scott and Sagliocco (1991a, b) considered *P. chrysidiformis* to be as effective a biological control agent as *S. doryliformis*, but attempts to adjust its life cycle to southern hemisphere conditions failed and therefore their work on *P. chrysidiformis* was discontinued. Preliminary studies have been initiated at CABI Europe-Switzerland, aiming to further study the biology of *P. chrysidiformis* and to develop rearing protocols. Mass-rearing and release methods have been developed in Australia for *S. doryliformis* (Fisher, 1992), using pieces of dock root for larval rearing, and gluing eggs to swizzle sticks by machine and inserting them directly into cut flowering dock stems. We believe that these methods could easily be adapted to rearing *P. chrysidiformis*. In the UK, *P. chrysidiformis* occurs on *R. crispus* only in a couple of sea-cliff and shingle beach sites in Kent, SE

England, and is protected by law as an endangered species under Schedule 5 of the Wildlife and Countryside Act (1981). Thus, working with this species in the UK will be especially challenging, but the combination of insect conservation and weed biological control, if successful, would be particularly rewarding.

Two other sesiids, *P. minianiforme* Freyer and *S. triannuliformis* Freyer coexist on *Rumex* spp., especially *R. crispus* in SE Europe, with the latter species extending into north and central Europe (Grossrieder and Keary, 2004). The biological control potential of both should also be considered.

## Fungi

Three species of pathogenic fungi commonly infect weedy *Rumex* spp. throughout Europe, and have potential for their biological control.

The rust *Uromyces rumicis* (Schum.) Wint. is the most studied fungus on *Rumex* spp., and was considered in the 1960s as a potential biological control agent for *R. crispus* in the USA (Inman, 1970). However, work was discontinued when it was impossible to confirm the alternate hosts of the fungus [in Europe the fungus is almost entirely spread through uredospores and teleutospores, but rarely forms spermogonia and aecidia on *Ranunculus ficaria* L. as an alternate host (Schubiger *et al.*, 1985)]. *U. rumicis* can cause up to 35% reduction in dry weight of *R. obtusifolius* during the first year of growth (Hatcher *et al.*, 1997), up to 60% shoot and 52% root reduction of *R. crispus* first-year overwintering plants (Hatcher, 1996), and can cause up to 40% reduction of dry mass in the first four years of *R. obtusifolius* growth in the field (Hatcher, unpublished data.). *U. rumicis* damage is not normally apparent in the field until late in the year, after dock has flowered, and thus it usually has little effect on seed production. It also cannot infect young developing dock leaves and as it is non-systemic, the plant is able to outgrow fungal damage (Hatcher *et al.*, 1995). However, while *U. rumicis* is not promising as a sole biological control agent for *Rumex* spp., it combines well with *G. viridula*. The rust infects the older leaves, causing the beetles to move to the younger leaves; thus an additive amount of damage is consistently produced by combined beetle and rust attack (Hatcher, 1996; Hatcher *et al.*, 1997; Hatcher and Paul, 2001). Artificial inoculation with the rust early in the year is possible, and in cool, moist climates is likely to persist over much of the summer. It is easy to produce large numbers of uredospores for artificial inoculation from *R. obtusifolius* plants in the laboratory or glasshouse (Hatcher *et al.*, 1994; Hatcher, 1996).

The necrotrophic fungus *Ramularia rubella* (Bon.) Nannf. also shows promise as a dock biological control agent. Unlike *U. rumicis,* this fungus can be cultured on agar and thus might be bulked up in the laboratory but there has been insufficient work to ascertain whether this is likely to be easy or not. It also occurs early, grows throughout the year on dock, and can cause almost total defoliation—including younger leaves—during severe outbreaks (Hatcher, personal observation). This fungus is very common on *R. longifolius* in Norway. Hüber-Meinicke *et al.* (1989) found that infection by this fungus reduced shoot weight of *R. obtusifolius* by 58% after 11 weeks, and root weight by up to 48%.

*Venturia rumicis* (Desm.) Wint. also occurs on *R. crispus* and *R. obtusifolius*. This hemibiotrophic ascomycete is the least common of these three pathogens in the UK and usually causes damage in late summer and autumn, although low levels can be present throughout the year. It is unclear whether this fungus can be cultured *in vitro* and no work has yet been attempted on using it as a biological control agent.

# The way forward

Along with meeting all the usual criteria for biological control agents, agents selected for dock biological control in organic agriculture must also be able to cope with the cultural control methods already being practiced by organic growers until and unless they can be demonstrated to be superior to these. Thus, the interactions among and between insect and fungus species become important, and also the effects of cutting, grazing and other control methods on the population dynamics of the insects and pathogens need to be studied *in situ*, under a range of conditions. For example, *P. chrysidiformis* oviposits on dry dock stalks, so any attempt to 'clean' dock-infested pastures by cutting the dry stalks may seriously hamper the population buildup of this moth.

Recent reviews of potential dock biological control agents (Grossrieder and Keary, 2004; Zaller, 2004; Bond *et al.*, 2006) suggest that of the indigenous species, *Gastrophysa viridula* and *Uromyces rumicis* still show the greatest promise for inundative biological control at the moment, but that *Ramularia rubella* and *Brachycaudus rumexicolens* need further investigation to determine their potential.

One way forward is to engage organic growers in enhancing or conserving the herbivores and pathogens they already have on *Rumex*. This has started in the UK with the HDRA-managed project mentioned above. A questionnaire about *G. viridula* received 34 replies: 23 respondents had seen the beetle, and 18 said that it occurred every year and noted that it caused significant damage to the plant, although this could be patchy. Ideas for encouraging the beetle in field margins at the start of the growing season by covering plants with fleece or simple polytunnels, for example, were put forward. It is easy to rear the beetle in bulk, either in plastic boxes on detached dock leaves (the beetle pupates under several layers of absorbent paper at the bottom of the box) or on plants grown in pots and sleeved with

perforated plastic bags. Both methods have been used for many years at the University of Reading, UK, and can produce many thousands of gravid female beetles (thought to be the best stage for release). This has enabled populations to be introduced into new areas of southern England, which have established after introduction (Hatcher, personal observation). Such methods could easily be adopted by farmers and thus inundative and conservation biological control could be practised with this insect.

As mentioned above, the beetle can be combined with other biocontrol agents, and a combination of *G. viridula* and *U. rumicis* with early re-sowing of *Lolium perenne* L. can control the flush of emerging *R. obtusifolius* seedlings after a pasture seed-bed is prepared (Keary and Hatcher, 2004). It is possible that regular cutting of dock-infested grassland may inhibit *G. viridula,* for example if it occurs during a peak egg-laying period. However, natural populations of the beetle are rarely synchronized and the beetle has persisted throughout a ten-year experiment at the University of Reading, UK, in a field which is mown at least four times a year without regard for the beetle (Hatcher, personal observation). It is also possible to modify mowing regimes to accommodate the beetle. In Austria, Hann and Kromp (2003) found that 'beetle-friendly' mowing (mowing twice per year rather than the three times used in conventional management, and each mow timed to coincide with the period the beetle was in the soil as a pupa) had a positive effect on *G. viridula* density and feeding damage, compared to a conventional regime of three cuts per year. The beetles were able to spread over the site, and unmown sites had greater numbers of overwintering adults than the mown ones. Thus, unmown refuges at the edge of fields could be useful for the beetle.

However, as we noted above, *Rumex* spp. are resilient to defoliation and have fast regrowth rates due to tap-root reserves that are rapidly replenished after regrowth of leaves. Hence, successful control strategies should include organisms that target other parts of the plant than the foliage, in particular the below-ground storage organs. Further research on native European insects feeding on dock roots, such as the clearwing moth *P. chrysidiformis*, may be fruitful and could provide, alone or in combination with defoliating organisms, effective biological control options. Additional control organisms and combinations of effective control agents also need further investigation to determine their suitability for mass-rearing and their potential to reduce dock populations in European organic agriculture.

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# Opportunities for classical biological control of weeds in European overseas territories

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*Summary*

European overseas territories are home to biodiversity and endemism of worldwide importance, vastly superior to that of continental Europe as a whole. They are, however, much more threatened by invasive species, including hundreds of alien invasive plant species having a huge impact on natural and agricultural habitats. As in continental Europe, invasive plants have only recently been recognized as a threat to the local environment and biodiversity. Mechanical and chemical control programmes—underway for several decades—have not been entirely successful for permanent, cost-effective, environment-friendly management. Biological control of weeds has long been successfully used in other neighbouring countries with similar climates, environmental conditions and invasions, but has barely been implemented in European overseas territories. There have been very few attempts to set up classical biological control programmes in these regions—a few of the species that have been the focus of biological control are *Lantana camara* L., *Rubus alceifolius* Poir., *Opuntia stricta* (Haw.) Haw., *Acanthocereus tetragonus* (L.) Britton & Rose, *Ligustrum robustum* (Roxb.) Blume, *Miconia calvescens* DC., *Ulex europaeus* L., *Prosopis juliflora* (SW.) DC., and *Leucaena leucocephala* (Lam.) de Wit. Many invasive plants occurring in European overseas territories are also invasive elsewhere and already targets of biological control programmes. Biological control agent specificity requires particular attention due to the high level of endemism in such islands. This paper reviews some of the most threatening species for which classical biological control could be achieved through regional or international collaboration.

***Keywords***: tropical island, invasive plants, biological control agent.

## Introduction

It is well known that invasive alien species are considered to be one of the greatest threats to biodiversity after habitat degradation, particularly in island ecosystems. European overseas territories consist of seven Ultra-Peripheral Regions (UPRs) that are an integral part of the European Union and 21 Overseas Countries and Territories (OCTs) that benefit from a system of close association (Table 1). Hereafter these two groups are jointly referred to as European Overseas Regions and Territories (EORTs). These EORTs are home to biodiversity of worldwide importance and vastly superior to that of continental Europe as a whole. Three French UPRs and 13 OCTs are involved in four of the 34 world biodiversity 'hotspots' (Conservation-International, 2006; Mittermeier *et al.*, 2005). R.A. Mittermeier, President of Conservation International, stated that the most remarkable places on Earth are also the most threatened, and it is in these territories that the speed of species extinction is the fastest worldwide. These territories have also hosted many species introductions—mainly plants, some of which have become invasive. For instance, over the last 300 years,

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**Table 1.** European Overseas Regions and Territories selected according to their climate.[a]

| European Overseas Regions and Territories | Country | European status | **Climate** |
|---|---|---|---|
| Azores | Portugal | UPR | warm temp./subtrop. |
| Canaries | Spain | UPR | warm temp./subtrop. |
| Guadeloupe | France | UPR | tropical |
| French Guiana | France | UPR | tropical |
| Madeira | Portugal | UPR | warm temp./subtrop. |
| Martinique | France | UPR | tropical |
| Réunion | France | UPR | tropical/temperate |
| Anguilla | United Kingdom | OCT | tropical |
| Aruba | Nederland | OCT | tropical |
| BAT (British Atlantic Territories) | United Kingdom | OCT | temperate |
| Bermuda | United Kingdom | OCT | tropical |
| BIOT (British Indian Ocean Territories) | United Kingdom | OCT | tropical |
| British Antarctic | United Kingdom | OCT | polar |
| BVI (British Virgin Islands) | United Kingdom | OCT | tropical |
| Cayman | United Kingdom | OCT | tropical |
| Greenland | Danmark | OCT | polar |
| Mayotte | France | OCT | tropical |
| Montserrat | United Kingdom | OCT | tropical |
| Nederland Antilles | Netherlands | OCT | tropical |
| New Caledonia | France | OCT | tropical |
| Pitcairn | United Kingdom | OCT | tropical |
| French Polynesia | France | OCT | tropical |
| Saint Pierre et Miquelon | France | OCT | polar/temperate |
| St Helena (+ Ascencion, Tristan da Cuña) | United Kingdom | OCT | temperate/tropical |
| TAAF (Terres Australes et Antarctiques Françaises) | France | OCT | polar/temperate |
| Turks & Caïcos | United Kingdom | OCT | tropical |
| Wallis and Futuna | France | OCT | tropical |

[a] European Overseas Regions and Territories shaded in grey were not considered in the study.

2217 plant species have been introduced on Réunion Island, 628 have become naturalized, and 62 were considered as invasive in the 1990s (Gargominy, 2003; Macdonald *et al*., 1991). There are currently around 200 invasive plant species. For all the French overseas territories, Gargominy (2003) highlighted the negative role of invasive species with respect to biodiversity conservation. Weed control in EORTs is essentially mechanical and/or chemical (Hivert, 2003) and never succeeds in long-term regulation of populations (Brondeau and Triolo, 2007). Eradication appears to be an efficient way (technically and economically) to control aliens on islands but requires early invader detection and rapid political decision-making before the plant has time to spread throughout a large area (Loope *et al.*, 2006). Only a few biological control programmes have been implemented in the EORTs, all of which were local programmes without any between-EORT collaboration. In this paper, we analyse exotic flora of EORTs to identify species common to several EORTs. We selected five species among those present in more than five EORTs that are under efficient classical biological control in other parts of the world. Here we present classical biological control programmes that could be implemented as European collaborative actions between EORTs and international collaborations with other countries that have already successfully directed such control programmes.

## Methods and materials

EORT climates range from polar to tropical, according to their geographical location. We selected EORTs with warm temperate, subtropical and tropical climates for this analysis. The degree of EORT invasion by alien plants was analysed on the basis of literature data and personal knowledge of certain situations (e.g. Réunion, New Caledonia, French Polynesia). A list of alien invasive species in EORTs was compiled from several databases, literature and ongoing synthesis projects in UK overseas territories (Varnham, 2005), the Canaries (Sanz-Elorza *et al.*, 2005), Madeira (Medeiros, 2006), Azores (Silva, pers. comm.) (Silva and Smith, 2004), Antilles (Joseph, 2006), French Polynesia (Meyer, 2000, 2004), New Caledonia (de Garine-Wichatitsky *et al.*, 2004; Meyer *et al.*, 2006), the Caribbean region (Kairo *et al.*, 2001) and the IUCN database of invasive species in French overseas territories, (Soubeyran, unpublished data). A species/EORT matrix was built. The nomenclature of plant species was verified according

to the Global Compendium of Weeds (Randall, 2002). We analysed the number of species mentioned in several EORTs. Species present in five or more EORTs were selected. We compiled plant biological control research or action programmes implemented in EORTs, and species that are already under biological control in other countries (Julien and Griffiths, 1998). We considered the possibility of developing a biological control programme through collaborations between EORTs for each invasive species.

# Results

## Plant invasions in EORTs

From seven UPRs and 21 OCTs, we selected 22 EORTs with warm temperate, subtropical, or tropical climates (Table 1). Saint Helena, Ascension and Tristan da Cuna were considered as three different entities, which means we included 24 different sites in this study. A list of 1267 plant species was compiled from invasive plant lists for the different EORTs. The number of plants per site ranges from three for French Guiana and Aruba to 410 for Bermuda (Table 2). There are two explanations for this variation. The first explanation concerns the origin of the information. In some lists, only environmental weeds are considered to be the most important invasive species, while both environmental and agricultural weeds are taken into account in other lists. The second explanation is that EORT invasion patterns differ markedly between sites. For instance, Joseph (2006) recorded very few invasive plants (22) in Martinique compared to Réunion (178). It is also well known that continental sites such as French Guiana are less invaded than oceanic islands. We found 75 species that invaded at least five sites (Table 3). *Leucaena leucocephala* (Lam.) de Wit (recorded at 21 different sites) appears to be the most common and best-distributed species. Five other species are present at 10 sites at least (*Lantana camara* L., *Psidium guajava* L., *Albizia lebbeck* (L.) Benth., *Casuarina equisetifolia* L., *Ricinus communis* L.). There are about 851 and 205 species present at only one or two sites, respectively. Most of them are common weeds present in other EORTs, but are not considered as invaders or environmental threats and are thus not listed. However, some of them, even though they are only considered to be invasive at one site, are highly invasive, e.g. *Hiptage benghalensis* (L.) Kurz, which seriously threatens local vegetation in dry habitats of Réunion, and the small tree *Miconia calvescens* DC. in the French Polynesian rainforest.

## Biological control programmes in EORTs

Only a few classical biological control research or action programmes of have been undertaken despite the extent of the invasive plant problems in most EORTs.

**Table 2.** Number of alien, invasive weeds per European Overseas Regions and Territories (EORT).

| EORT | Number of weeds |
|---|---|
| F French Guiana | 3 |
| NL Aruba | 3 |
| NL Netherland Antilles | 7 |
| UK Turk & Caicos | 8 |
| P Madeira | 10 |
| P Azores | 12 |
| UK BVI | 15 |
| F Guadeloupe | 18 |
| F Martinique | 22 |
| UK Pitcairn | 26 |
| UK Montserrat | 28 |
| UK Tristan da Cuna | 49 |
| F New Caledonia | 67 |
| F Wallis Futuna | 61 |
| UK Cayman | 74 |
| F French Polynesia | 96 |
| UK Ascension | 101 |
| ES Canaries | 151 |
| F Réunion | 178 |
| F Mayotte | 190 |
| UK Anguilla | 196 |
| UK BIOT | 230 |
| UK St Helena | 288 |
| UK Bermuda | 410 |

The first one was launched in the early 1900s, with the introduction and release of *Ophiomyia lantanae* (Froggatt) for *L. camara* control in French Polynesia (1916) and New Caledonia (1924). Then four other agents (*Teleonemia scrupulosa* Stal, *Syngamia haemorrhoidalis* Guen., *Octotoma scabripennis* Guérin-Méneville, *Uroplata girardi* Pic.) were released on this island over the next 50 years, with varying degrees of efficacy against *L. camara* (Gutierrez, 1976, 1979). This plant has also been biologically controlled in other places (Saint Helena, Ascension) (Julien and Griffiths, 1998). Finally, only seven EORTs have developed a biological control programme (New Caledonia, French Polynesia, Saint Helena, Ascension, Réunion, Montserrat and Cayman) and only nine plant species have been considered for biological control research programmes or release, including: *L. camara* (see above); *Opuntia stricta* (Haw.) Haw. (New Caledonia, Cayman), *O. triacanthos* (Willd.) Sweet (Montserrat) and *Opuntia* sp. (New Caledonia, Saint Helena, Ascension), using *Cactoblastis cactorum* (Berg) with good success; *Acanthocereus pentagonus* (L.) Britton & Rose (New Caledonia), using *Hypogeococcus festerianus* (Lizar & Trelles); *Miconia calvescens* DC. (French Polynesia), using *Colletotrichum gloeosporioides* L. f. sp. *miconiae*; *Rubus alceifolius* Poir. (Réunion), using *Cibdela janthina* (Klug); *Ulex europaeus* L. (Saint Helena), us-

**Table 3.** List of weed species considered invasive at five sites at least.

| Species | Total |
|---|---|
| *Leucaena leucocephala* | 21 |
| *Lantana camara* | 13 |
| *Psidium guajava* | 12 |
| *Albizia lebbeck* | 11 |
| *Casuarina equisetifolia* | 11 |
| *Ricinus communis* | 10 |
| *Acacia farnesiana* | 9 |
| *Argemone mexicana* | 9 |
| *Bryophyllum pinnatum* | 9 |
| *Melaleuca quinquenervia* | 9 |
| *Panicum maximum* | 9 |
| *Schinus terebinthifolius* | 9 |
| *Eicchornia crassipes* | 9 |
| *Cynodon dactylon* | 8 |
| *Commelina diffusa* | 8 |
| *Mirabilis jalapa* | 8 |
| *Solanum mauritianum* | 8 |
| *Tabebuia heterophylla* | 8 |
| *Tecoma stans* | 8 |
| *Pinus caribaea* | 8 |
| *Catharanthus roseus* | 7 |
| *Furcraea foetida* | 7 |
| *Melia azedarach* | 7 |
| *Canna indica* | 7 |
| *Syzygium jambos* | 7 |
| *Achyranthes aspera* | 6 |
| *Agave americana* | 6 |
| *Ageratum conyzoides* | 6 |
| *Antigonon leptopus* | 6 |
| *Bidens pilosa* | 6 |
| *Chamaesyce hirta* | 6 |
| *Cyperus rotundus* | 6 |
| *Grevillea robusta* | 6 |
| *Oxalis corniculata* | 6 |
| *Passiflora suberosa* | 6 |
| *Pennisetum purpureum* | 6 |
| *Pittosporum undulatum* | 6 |
| *Prosopis juliflora* | 6 |
| *Solanum nigrum* | 6 |
| *Spathodea campanulata* | 6 |
| *Terminalia catappa* | 6 |
| *Urochloa mutica* | 6 |
| *Ziziphus mauritiana* | 6 |
| *Adenanthera pavonina* | 5 |
| *Agave sisalana* | 5 |
| *Asclepias curassavica* | 5 |
| *Bambusa vulgaris* | 5 |
| *Carpobrotus edulis* | 5 |
| *Cenchrus echinatus* | 5 |
| *Clidemia hirta* | 5 |
| *Conyza bonariensis* | 5 |
| *Cryptostegia grandiflora* | 5 |
| *Eleusine indica* | 5 |
| *Eriobotrya japonica* | 5 |
| *Leucaena diversifolia* | 5 |
| *Manilkara zapota* | 5 |
| *Melinis repens* | 5 |
| *Mimosa pudica* | 5 |
| *Momordica charantia* | 5 |
| *Opuntia ficus-indica* | 5 |
| *Paspalum conjugatum* | 5 |
| *Passiflora foetida* | 5 |
| *Phoenix dactylifera* | 5 |
| *Physalis peruviana* | 5 |
| *Plantago major* | 5 |
| *Psidium cattleianum* | 5 |
| *Rubus rosifolius* | 5 |
| *Senna occidentalis* | 5 |
| *Sida acuta* | 5 |
| *Sorghum halepense* | 5 |
| *Sphagneticola trilobata* | 5 |
| *Sporobolus indicus* | 5 |
| *Stachytarpheta urticifolia* | 5 |
| *Tamarindus indica* | 5 |
| *Ulex europaeus* | 5 |

ing *Tetranychus lintearius* Dufour; *Prosopis juliflora* (Sw.) DC. (Ascension), using *Heteropsylla reducta* Caldwell & Martorell (and *Rhinochloa* sp. accidentally introduced); and *Ligustrum robustum* (Roxb.) Blume subsp. w*alkeri* (Decne.) P.S.Green (Réunion), for which *Epiplema albida* (Cassino & Swett) has been tested but not yet released (CABI c.p., Julien and Griffiths, 1998; Meyer, 1998). The situation concerning *L. leucocephala* is interesting. This plant is considered as invasive almost everywhere it occurs and is the most widely distributed species throughout all EORTs. From 1985 to 1991, *Heteropsylla cubana* Crawford, a biological control agent, arrived naturally or accidentally in French Polynesia, New Caledonia, and later in Réunion and subsequently controlled this invasive plant. Because of a conflict of interest regarding this invasive species, which is also a forage plant, it was decided to biologically control *H. cubana* using the lady bird beetle *Olla v-nigrum* (Mulsant) (Chazeau *et al.*,1989; Quilici *et al.*, 1995). Some other biological control actions were also accidental, e.g. *Rhinochloa* sp. against *P. juliflora* in Ascension. For others, such as *U. europaeus* in Saint Helena, the biological control agent *T. lintearius* was introduced along with its predator *Phytoseiulus* sp., thus nullifying the biological control. Most biological agents released were arthropods. The only pathogen was *C. gloeosporioides* f.sp. *miconiae* for control of *M. calvescens* in French Polynesia (Meyer and Killgore, 2000). This review highlights the very low number of biological control actions undertaken in EORTs despite the fact that invasive plants are

highly numerous and damaging to the environment and biodiversity. Nevertheless, many of these species are already targets of biological control actions or research in other parts of the world.

## Biological control programmes that could be implemented in different EORTs

Cochereau (1972), proposed several strategies for classical weed biological control programmes in the Pacific, including targets such as *Psidium guajava* L., *Melaleuca quinquenervia* (Cav.) T.Blake, *Elephantopus mollis* Kunth, *Stachytarpheta jamaicensis* (L.) Vahl, *Mimosa invisa* C.Mart. ex Colla *, L. leucocephala, Solanum torvum* Sw.*, Cyperus rotundus* L.*, Rubus rosifolius* Sm.*,* and *Ageratum conyzoides* L. Developing a new biological research programme (without any knowledge of the target plant or its natural enemies) is a very long process (more than 10 years) and very expensive with regard to a typical EORT budget, whereas transferring biological control technology from countries where programmes are already underway is much more time- and cost-effective. As many invaders are common to several EORTs, joint biological control programmes could easily be implemented at a European-overseas level. If EORTs decide to work together to solve the problem of alien plants, species should be selected that are common to several sites. We have noted that 78 plant species are invasive at five or more sites. It is clearly not possible to implement so many biological control programmes and most of these species are not yet biologically controlled elsewhere in the world. To illustrate opportunities for developing classical biological control actions in EORTs, we selected five species according to four criteria: (1) historical success of biological control of this target in other countries with ecological similarities, (2) taxonomic isolation of these weeds from indigenous flora in EORTs, (3) good knowledge of biological control agents that are suitable for use in EORTs, and (4) species that are not sources of any conflicts of interest, such as *Schinus terebenthifolius* Raddi for honey or spice production, *Psidium catleianum* Sabine and *P. guajava* for fruit production, or *Acacia* spp. for wood production. The authors understand that this selection cannot be considered a priority for every EORT, as each one has its own priorities in controlling invaders and/or biodiversity conservation. Nevertheless, the common feature of the following five examples is that they could be implemented easily, rapidly, with a high probability of success, and at low cost.

**Case 1: *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae):** Nine sites are affected (Canaries, Guadeloupe, Martinique, French Polynesia, New Caledonia, Réunion, Bermuda, BVI, Cayman). Water hyacinth is widely recognized as the world's worst aquatic weed. Native to the Amazon basin, it was exported throughout the tropics and warm temperate regions for its flower and for water treatment. It forms dense mats on water bodies, thus limiting access to water, navigation, and fishing. It produces $H_2S$ in the water, reduces the water pH, increases evaporation, and reduces light penetration and oxygen content. This leads to dramatic biological changes, with social and economic consequences. Physical and chemical controls are very expensive, temporary, and ecologically and economically unsustainable. Classical biological control is the only feasible way to manage such widespread infestations. A number of biological control agents have now been introduced in about 30 countries. The species most widely used are *Neochetina* weevils, *N. bruchi* Hustache and *N. eichhorniae* Warner (Coleoptera, Curculionidae) (Julien *et al.*, 1999). With a 30-year history, the biologies, host ranges, rearing, release and monitoring techniques are well documented (Julien *et al.*, 1999), and the efficiency is fully recognized in many countries. Other agents are also used, such as the two moths, *Niphograpta albigutalis* Warren and *Xubida infusellus* (Walker) (Lepidoptera, Pyralidae), which have been released in 13 and three countries, respectively (Julien *et al.*, 2001). The weevils are currently reared in South Africa at PPRI and can be considered as the most suitable agents to initially release on tropical islands, with an expected high success rate within two to seven years (Le Bourgeois and Lebreton, 2006).

**Case 2: *Ulex europaeus* L. (Fabaceae):** Five sites are affected (Canaries, Réunion, Azores, Ascension, Saint Helena). Native to the Western coast of Europe (UK, France, Portugal), gorse is a prickly, perennial, evergreen legume which grows up to 3 m in height. It reproduces mainly by seed and is spread by machinery, soil movements, water and animals. It is a major weed problem in pastures and natural habitats, increasing the risk of brushfires, reducing land utilization by forming dense thickets, dramatically reducing stocking rates and competing with native species of subalpine shrublands. It is considered as a weed of national significance in Australia, New Zealand and Hawaii. Several biological control agents have already been used for gorse. The gorse seed weevil *Exapion ulicis* Forst. (Coleoptera, Curculionidae) was introduced into Australia in 1939 after being released in New Zealand. Its impact is limited because the larvae are not present during the second period of seed production. In 1998, the gorse spider mite *T. lintearius* was released in Australia and New Zealand. It forms colonies on plants and spin a tent-like white web and feed on the leaves and branches. This spider mite may have a substantial impact but is regulated by other mites such as phytoseids (Acari, Phytoseidae). Other agents are under study, including the gorse thrips *Sericothrips staphylinus* Haliday. In Monserrat, the introduction of *T. lintearius* had no impact on gorse populations of the island, likely due to the concomitant, accidental introduction of its predator, *Phytoseiulus* sp. Pure populations of such biological control agents must be introduced from the beginning

and studies should be conducted to determine if indigenous phytoseids already exist in the area of introduction (Anonymous, 2003; Davies *et al.*, 2004; Krause *et al.*, 1988).

**Case 3: *Clidemia hirta* (L.) D.Don (Melastomataceae):** Five sites are affected (Canaries, Mayotte, Réunion, Wallis and Futuna, Ascension). Koster's curse is native to tropical America (Mexico and the West Indies, and southward to central Brazil). This noxious weedy shrub grows up to 2 m tall in pastures and open forests. It is an aggressive invader which shades out all underlying vegetation. The seeds are principally dispersed by frugivorous birds but any organism moving through the thickets will carry seeds away with it. It is probably not resistant to fire, which is unlikely in its habitat, but it rapidly colonizes burned areas. Introduced in Réunion during the 1970s, it now colonizes the wet forest understorey on the southeast coast and roadsides and agricultural fields on the east coast. Several expeditions to find potential biological control agents have been carried out in Trinidad, and a number of insects were collected and screened. In Hawaii, a thrips, *Liothrips urichi* Karny (Thysanoptera, Phlaethripidae), which was introduced in 1953, works well in open areas but not in the shade of forests; while the fungus, *Colletotrichum gloeosporioides* (Penz) Sace. f.sp. *clidemiae* (Coelomycetes, Melanconiales), introduced in 1986, is efficient in shady and wet places. Both the insect and pathogen would be complementary in Réunion and Mayotte settings. The Hawaii Department of Natural Resources and University of Hawaii are still testing other agents such as *Lius poseidon* Napp, a beetle; and moths *Antiblemma acclinalis* Hubner, *Carposina bullata* Meyrick and *Mompha trithalama* Meyrick (Julien and Griffiths, 1998; Nakahara *et al.*, 1992; PIER, 2006a; Trujillo, 2005)

**Case 4: *Pistia stratiotes* L. (Araceae):** Four sites are affected (Martinique, Réunion, Bermuda, New Caledonia). Its origin is unknown, but it is now pan-tropical. Water lettuce is a common aquatic weed in countries with hot climates. It is a floating, rosette-forming, stemless, stoloniferous herb. This plant usually propagates by means of stolons which break easily from the plant and it also propagates by seed. It causes similar problems as *E. crassipes* on bodies of water. The weevil *Neohydronomous affinis* Hustache (Curculionidae, Erirhinae), which was collected in South America, substantially reduced growth of *P. stratiotes* in Australia and Zimbabwe. It has now spread to more than six countries. This is the most sustainable method to control this free-floating weed. It has been readily established in six countries and has provided substantial to excellent control in all of them. For introduction in Réunion, *N. affinis* can be obtained from PPRI in South Africa (DeLoach, 1978; Dray and Center, 2003; Foxcroft and Richardson, 2003).

**Case 5: *Ageratina riparia* (Regel) R.M.King & H.Rob. (Asteraceae):** Three sites are affected (Canaries, Réunion, Bermuda). Mistflower is native to Mexico. It is a low-growing perennial with tiny, white, daisy-like flowers. It rapidly invades disturbed areas and tends to spread along gullies and river banks. It is rather a hemisciaphilous species which is confined to the forest margins, paths, and gullies in subtropical to temperate climates. Chemicals from its leaf-litter suppress the growth of other plants, giving mistflower a further competitive advantage. A plume moth, *Oidematophorus beneficus* Yano & Heppner (Lepidoptera, Pterophoridae), a gall wasp, *Procecidochares alani* Steyskal (Diptera, Tephritidae) and a smut fungus, *Entyloma ageratinae* Barreto & Evans (Ustilaginales, Basidiomycotina), were introduced in Hawaii to attack this aggressive weed in the mid-1970s. Biological control of mistflower in Hawaii has been an outstanding success. Of the three agents, the fungus was the most effective and it achieved total control of the plant in wet areas within 8 months, and in dry areas within 3–8 years. The plant has remained under control ever since. Mistflower has increasingly become a problem in northern New Zealand. A feasibility study showed that infested areas of New Zealand were likely suitable for the mistflower agents, so the smut fungus and the gall wasp were released in New Zealand in 1998 and 2001, respectively. Both are establishing and spreading rapidly and it looks promising that the plant will be successfully controlled there too. Technology transfer from Hawaii or New Zealand to Réunion, Canaries or Bermuda would be easy. In Réunion, no endemic species belonging to the Eupatoriae tribe are present, and there are only two indigenous species of the *Adenostemma* genus—these should be tested to determine the specificity of the potential agents (Frohlich *et al.*, 2000; Morin *et al.*, 1997; PIER, 2006b).

# Conclusion

Biodiversity is more threatened by alien invasive plants in tropical European overseas departments, territories, and countries than in continental European countries but very few classical weed biological control programmes have been undertaken to date. Many invasive species are common to several EORTs and many of them are already under biological control programmes in other countries. These biological control agents and technologies could be easily transferred within collaborative European and international programmes. Prior to any introduction, a review of host-specificity test results is necessary to determine whether complementary tests should be done according to the indigenous or endemic flora conservation concerns in each setting. We have described some examples of invasive species that could be controlled with a high probability of success by several existing and proven biological control agents, such as *E. crassipes, U. europaeus, C. hirta, A. riparia,* and *P. stratiotes*. Many other weed species

could also be targeted in EORTs by classical biological control throughout inter-EORT collaboration, e.g. *C. gloeosporioides* f.sp. *miconiae* from French Polynesia to control *M. calvescens* at an early invasion stage in New Caledonia and the Canaries, or through international collaborations (e.g. with Hawaii). EORTs should be highly suitable places to implement classical biological control of alien invasive plants.

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# Weed biological control regulation in Europe: boring but important

**R.H. Shaw**[1]

*Summary*

If the Europe Union (EU) is to deliver on its Convention on Biological Diversity (CBD) commitments and its Member States are to have any chance of achieving a good status classification for their water bodies, classical biological control will be needed. A lack of history and considerable inertia impede the development of biological control programmes but an inappropriate and ineffective regulatory environment could prove more of a barrier to weed biological control implementation. This paper reviews the current situation in Europe and considers the codes and regulations for the release of arthropods and fungi before considering the suitability of pest risk assessments.

***Keywords***: legislation, EU, pest risk assessment.

## Introduction

As signatories to the Convention on Biological Diversity, European Union Member States (MS) have an obligation to 'prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats and species' (Decision VI/23 in 1992). They are also encouraged to invest in research and assessment of biological control as a control option. The European Strategy on Invasive Alien Species (ESIAS) came in to being in 2003, and calls for a regional approach to the problem highlighting the need for cost: benefit analyses of long-term control measures. Exotic weeds are amongst the most problematic of invasive species but are amenable to biological control (Cruttwell McFadyen, 1998). In parallel many herbicides have been lost as a result of changes in registration requirements and there is no shortage of invasive plant species in Europe and more potential invasive alien weeds arrive daily. A recent review by EPPO recorded the shipments of aquatic plants into one French airport and revealed an average of more than one shipment per day of exotic plants. In the month of May 2006 alone, almost 100,000 plants arrived through this port (EPPO, 2007) including ten species that are known problematic invaders in Europe and another nine that are not yet present in Europe but known to be invasive elsewhere. As exotic species with a lack of specialist natural enemies, many of the invasive weeds in Europe would be amenable to classical biological control (Sheppard *et al.*, 2006) and some have 'off-the-shelf' agents that have been thoroughly tested elsewhere and are likely to be safe for release. It would seem that the stage is set for the expansion of classical biocontrol of weeds into Europe.

In much the same way as the weed invades, biological control activity could be expected to increase rapidly from a slow start, and the current lack of precedent could be a reason in itself for a lack of take-up. In reality, a major factor is public and political perception. Plants are perceived by the public and their representatives as less threatening than insect pests since the latter more obviously threaten our food supply. Thus, a proposal to engage in an irreversible introduction of yet another alien species can seem counterintuitive. Fear of what might go wrong is certainly a consideration when a new concept is proposed, but other factors may prove to be more important. Though public perceptions and governmental needs are changing, in part thanks to ongoing programmes against Japanese knotweed, *Fallopia japonica* (Djeddour *et al.*, these proceedings), the crucial regulatory environment is far from ideal.

## The regulations

The regulatory framework in Europe is characterised by a lack of clarity and accompanied by varied interpretation. The lack of history of weed biological control in Europe is almost certainly the cause of the legislative gaps and similar situations existed in more experienced

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countries prior to their development of tailored legislation such as the Australian Biocontrol Act 1986, and the New Zealand Hazardous Substances and new Organism Act 1996. Thus, many countries find themselves using regulations that were not designed for the purpose of dealing with the introduction and release of exotic biological control agents such as those provided for plant quarantine, genetically modified organisms and wildlife conservation. The responsible Government department is often unclear about how to proceed, and this was a significant factor in the failure to make positive decisions to release agents in the bracken, *Pteridium aquilinum* (L.) Kuhn, programme in the 1980s (S.V. Fowler, personal communication, 1998).

To assess the situation in Europe, a letter was sent out to the Plant Protection Department of each European country in June 2005 requesting information on their regulation of classical weed biological control agents in general and more specifically those relating to plant pathogenic fungi. Of the 25 recipients of the request, 11 responded. What became clear was that classical agents, both insect and fungal, cause considerable problems not only in practical regulatory terms but also in understanding. Some countries clearly had no understanding of the meaning and implications of classical biological control, let alone the regulatory paths for the two taxa. In some cases the responsible authority for initial import and actual release differed, as did the departments dealing with fungi and insects.

The confusion comes about because, even though the ultimate goal and strategy of classical biological control is the same whether insect or fungal agents are considered, they are regulated very differently by the EU, and each country has to fit the requirements of its own legislation. For example, one of the early milestones in a biological control programme is agreeing to the test plant list to be used during host range testing. This is carried out by the Technical Advisory Group (TAG) in North America, but in the UK, for example, there appears to be no mechanism for agreeing to such lists prior to the application for release.

## Arthropods

European directive 2000/29/EU protects Europe against the introduction and spread of 'blacklisted' known pests and harmful macroorganisms by their prohibition. However, any species not on the list (including all potential classical biological control agents and many potentially invasive plants) can therefore be introduced without any formal risk assessment. This suggests that there is no EU level provision for assessing releases of beneficial exotics.

However, each EU member state transposes and interprets the ED 2000/29/EU into their national legislation and as a result can, and should, develop regulatory procedures for the releases of non-indigenous macroorganisms such as arthropod classical biological control agents (Genovesi and Shine, 2004). Currently there remains a wide divergence in such regulatory requirements from virtually none in many EU countries, to an incipient risk assessment process being developed with appropriate regulatory bodies in, for example, the UK and Portugal—the two countries most advanced in developing classical weed biological control programmes. In the commercial world a lack of clear legislation is often exploited. However, that is generally not the case in the public-interest, public-investment research sector, and consequently, weed biological control releases are conspicuous by their absence.

There have been over a thousand releases of biological control agents for weeds worldwide (Julien and Griffiths, 1998) yet no full classical weed biological control programme has been carried out in any EU Member State. This contrasts strongly with the repeated use of exotic insects to control insect pests in Europe. The BIOCAT database (Greathead and Greathead, 1992 – updated to end-2004) lists a staggering 137 species that have been included in a total of 276 releases since 1901.This extensive use of exotic natural enemies in Europe has been poorly regulated in some countries and, as a result, has not been without problems. This is not surprising since, for example, of the 65 species to have been introduced since 1908 into Spain alone, only four are considered monophagous (Jacas *et al.*, 2006). Thus, it is highly likely that extensive non-target effects are occurring without being noticed. The recent problem with the predatory ladybird *Harmonia axyridis* (Pallas) (Majerus *et al.*, 2006) highlights the dangers and may be another factor to hinder the acceptance of classical weed biological control in Europe.

There is an inherently greater general precaution over releases of herbivorous vs entomophagous arthropods because, as in the United States, the release agency is likely to be legally responsible should any biological control agent cause economic loss or significant environmental damage (Delfosse, 2005; Miller and Aplet, 1993).

EU countries would be expected to use the EPPO standards on the safe use of biological control (EPPO, 2000) as well as and the newly revised international advisory 'Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Organisms Claimed to be Beneficial' otherwise called the International Standards for Phytosanitary Measures, publication No. 3 or ISPM 3 (IPPC, 2005) [reviewed by Genovesi and Shine (2004); Kairo *et al.*, (2003)]. These documents also highlight the need for consultation between relevant neighbouring countries. Interestingly, ISPM 3 recommends that the National Plant Protection Organization should conduct a pest risk assessment (PRA) either before import or before release of biological control agents and other beneficial organisms. It is also inclusive of fungal agents, i.e. non-formulated micro-organisms that are released with the expectation of establishment. It follows, therefore,

that any national regulatory procedures developed by EU member states for the release of classical biological control agents, as a result of EU directive 2000/29/EU, and based on ISPM 3, should be technically capable of including both macro- and microorganism agents.

Another organisation that became involved in the regulation of arthropod biological control agents was the Organisation for Economic Cooperation and Development who produced a set of guidelines (OECD, 2004). These guidelines are very thorough and deal with the likelihood of establishment and efficacy as well as direct non-target effects. Seeing the threat posed to the commercial biological control industry if these guidelines became the template for national regulation, the International Biocontrol Manufacturers' Association (IBMA) proposed that the International Organisation for Biological Control (IOBC) should oversee the harmonization of all these documents into one. This harmonization document was published in 2005 (Bigler *et al.*, 2005) with the stated aim of providing advice to the competent national authorities on the information required to perform risk assessments with respect to the import and release of an insect biological control agent (IBCA). Most of the guidelines remain applicable to weed biological control agents but these have been specifically excluded from consideration. The sections are divided as follows:

- Information requirements for the importation of non-native IBCAs for research
- Information requirements for the deliberate release of non-native IBCAs
  - not previously released
  - previously authorized for release
- Information requirements for the release of native IBCAs

Despite this mass of non-binding documentation it is yet to be shown how any nation will actually implement the guidelines in their national legislation when it comes to the release of an invertebrate classical weed biological control agent. Amazingly, the situation is even more bizarre when one considers plant pathogens.

## Plant pathogens

The regulatory framework that exists in the EU for the use of plant pathogens in classical biological control is officially driven by one central EU directive which effectively hinders and may actually prevent their use. Although aimed at minimizing the use of chemicals by regulating 'the placing of plant protection products on the market', the EU directive for chemical pesticide regulation 91/414/EU, as updated by Council Directive 2005/25/EU, has been written in such a way as to include, by default, microorganisms as classical biological control agents. For example, the definitions section of the directive begins with a reference to the form in which the products are supplied to the user and the whole process revolves around label claims. This is contrary to classical biological control as there is no supply to users and there are no labels. That no specific consideration was given to micro-organisms as classical biological control agents is perhaps not unexpected, since it is a technique with no history in Europe. The situation, however, has been described as totally inadequate as it hinders progress (Seier, 2005).

A highly-specific obligate plant pathogen, released once in order to provide permanent control of the target weed, generates no subsequent sales, but must still go through a regulatory assessment application designed for non-specific herbicides. Such applications require large amounts of scientific data inappropriate for the use of pathogens as classical biological control agents (e.g. mammalian toxicity and efficacy versus current chemical alternatives). In addition, the application costs are high, although some EU countries appear willing to reduce the costs of application. The UK currently has a Biopesticide Scheme with dossier assessment costs of about £23,000 (33,400 Euro) and allows a reduced data package. Nonetheless, these costs and requirements are even considered prohibitive to the commercial biological control manufacturers who are producing products with labels and generating subsequent income.

Classical biological control agents are normally used in line with the EU goal of reducing chemical inputs to the environment. Yet such agents are blocked by inadequacies in the legislation aimed at stricter assessment of new pesticides. The consequences of 91/414/EU are wide reaching and could prevent the use of plant pathogens in classical biological control in Europe, despite an impeccable worldwide safety record (Barton, 2004) and high levels of effectiveness (Charudattan, 2005).

Recent reviews of the 91/414/EU directive have separated consideration of chemicals from microorganisms but there remains a need for a new directive or for revisions to cover classical biological control agents. As this will take considerable time, an interim measure might be to indicate that Member States apply the directive only to 'formulated products', thereby distinguishing between those microorganisms considered for commercialization (i.e. those requiring labels with storage, application and safety information) and those considered as classical fungal agents to be released once, or a few times, in the public interest.

One glimmer of hope is the current EU-funded project looking at the regulation of biological control agents in Europe (REBECA). The groups involved come from all fields of biological control from scientists, through producers to regulators, and are expected to make recommendations to the Commission. At a recent REBECA microbials subgroup meeting it was accepted that classical weed biocontrol agents that are fungi should be excluded from 91/414 (B. Ritchie, 2006, personal communication). It will be a step forward if such a recommendation is accepted by the EU.

The Japanese knotweed programme (see Djeddour *et al.*, these Proceedings) may be the first to test the regulatory framework, since both an insect and a leafspot fungus are primary candidate agents. Communications with various government departments since the late 1990s have attempted to clarify the pathway for each type of agent with little progress until 2005. Then initial meetings were held with the Pesticide Safety Directorate through whom the UK Government's Department of Environment Food and Rural Affairs (Defra) implements 91/414. Negotiations continue with an interdepartmental committee which was established just to deal with this particular issue. All parties are taking a pragmatic approach to the issues and mutually agreeable and practical solutions are expected.

## Pest Risk Assessment (PRA)

As intimated above, it would seem that some version of a PRA will be favoured by EU Member States. Historically the treatment of potential crop pests has differed from that of uncultivated plants but these are now coming together in the key area of risk analysis (Baker *et al.*, 2005). The International Plant Protection Convention (IPPC) was signed in 1951 as a response to increasing pest invasions, in particular that of the Colorado potato beetle, *Leptinotarsa decemlineata* Say. The IPPC has since produced 21 International Standards for Phytosanitary Measures (ISPMs) which are recognized by the World Trade Organisation (WTO). If a pest is of potential economic importance then it is considered a quarantine pest and joins the lists of such organisms posted by nations and trading blocks. To avoid unnecessary barriers to trade, both the IPPC and WTO stipulate that quarantine status and controls can only be put in place once a real threat is identified through a pest risk analysis. The tools for this are ISPM2 (FAO, 1996) and ISPM 11 (FAO, 2001).

According to the ISPM 2 revision document, which is currently out for member country consultation, biological control agents 'are intended to be beneficial to plants or plant products without causing harm'. This is true from an ecosystem perspective but quite the opposite of the intention when one considers the target plant to which harm is most definitely intended. It goes on to say that, 'when performing a PRA or monitoring their release, the main concern is unanticipated harm to non-target organisms in the PRA area'. The most appropriate current tool for assessing the risks associated with a biological control release is probably ISPM 11, which was established in 2001 and was updated in 2004 to include analysis of environmental risk and living modified organisms (FAO, 2004). Unhelpfully, it refers to pests throughout which from a biological control perspective is confusing since biological control agents must then be called 'beneficial pests'. Nonetheless, the IPPC defines a pest as '*any species strain or biotype of plant, animal or pathogenic agent, injurious to plants or plant products*' (FAO, 2004). As such, classical biological control agents rest firmly in ISPM 11. Furthermore, in section 1.1.2 of the latest version of ISPM 11, it is stated that a PRA may be initiated if a request is made to import an organism.

The general requirements of a PRA are fairly similar country to country but since they are written for pests, much of the information required is not necessarily appropriate for weed biological control. An analysis of the questions posed found that half are inappropriate, mainly because they deal with the risk of the pest's arrival and the prospects for its control. It seems increasingly likely that this form of PRA will be applied to biological control agents, at least in the UK, and it will probably be under Plant Health regulations, as suggested above. This is much the same as the situation in South Africa where the Directorate of Plant Health and Quality regulates any biological control agent release.

# Discussion

Biological control of weeds in Europe is just beginning and has all the teething troubles associated with such a period. The unclear funding and regulatory situation coupled with a general inertia has hindered the development of classical biological control and in some areas Europe reflects the situation in countries such as Australia and New Zealand as it was decades ago. Europe has an advantage in that it can learn from history and avoid the painful mistakes made by our pioneering ancestors, especially since a lot of biological control expertise is already present in European countries.

It is clear that at least those regulations governing fungal biological control agents are in need of revision. My proposal to precede description of Plant Protection Product with the word 'formulated' in the 91/414 Directive could solve most of the problems surrounding the presumably mistaken inclusion of classical fungal weed agents. As is often the case with authorities, simply trying to avoid regulations by requesting an exemption is not the best course, and the inclusion into Plant Health legislation would seem to be the best solution. However, it is likely that the early applications will need to be considered by all interested parties in the absence of tailored regulations.

As Europe comes to terms with its CBD commitments and the growing scale of invasions by environmental weeds, classical biological control should become more commonplace. This is particularly so in aquatic and riparian systems, where most of Europe completely bans the use of chemical herbicides and no real alternative exists. The driving force in such delicate habitats may well turn out to be the Water Framework Directive (Dec. 2000) which requires parties to ensure that all their waterways reach 'good status' by 2015. The presence of invasive alien species in or on

these waterways logically would prevent this goal being achieved and should focus attention on alternative approaches to their management, ones that are commonplace in countries in other continents—that is, classical biological control.

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# Field evaluation of *Fusarium oxysporum* as a biocontrol agent for *Orobanche ramose*

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Under the changing agro-climatic conditions of western Europe, the root parasitic weed *Orobanche ramosa* infests at a progressing rate host crops such as hemp, tobacco and to an increasing degree oilseed rape in France. *Fusarium oxysporum* (FOG) was isolated from *O. ramosa* tubercles, parasitizing tobacco in Germany. The fungus was formulated in wheat flour kaolin ('Pesta') granules and showed promising results in controlling *O. ramosa* under greenhouse conditions, reducing number and dry matter of the parasite by up to 90 %. Consequently FOG was tested under field conditions using different application techniques. In-furrow application and broadcasting of the inoculum after tobacco planting as well as subsoil application pre-planting decreased the number of *Orobanche* shoots. In a further experiment, in-furrow application of FOG markedly reduced number and dry matter of *O. ramosa*. However, no distinct further reduction could be noticed when biocontrol was combined with a resistance reducer. The results revealed the potential of plant pathogens for *Orobanche ramosa control* and future experiments should work on enhancing the observed effect under natural conditions.

# Potential for biological control of *Hydrocotyle ranunculoides* in Europe

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*Hydrocotyle ranunculoides* is an invasive aquatic macrophyte, present in several European countries and elsewhere outside its native range of southern North America. The plant has spread from single introductions to occupy marginal habitats of over 30 miles in at least three rivers in the UK in the last 4 years. Attempts have been made to control this plant using mechanical and chemical means, combined with manipulation of the environment. Most of these have proved unsuccessful due to rapid recolonisation. Given the restrictions on chemical use for aquatic weed control in Europe, we propose a novel combination of mechanical control and biological control as a technique to prevent the spread of the plant within catchments where it is already established, and to eradicate relatively new small infestations. Data will be provided on collection of potential biological control agents from Argentina, the initial screening and preliminary host specificity studies.

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# Alien poisonous weeds: a challenge for a biological control of weeds program in Europe

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Considering weeds as harmful organisms threatening ecosystems is acknowledged worldwide, however there are exotic weeds that have a second serious impact on human beings and livestock due to their high allergenic or poisonous capability. We review here the opportunity to apply a European biocontrol program against the North American ragweed (*Ambrosia artemisiifolia*) and silverleaf nightshade (*Solanum elaeagnifolium*), and the West-Asian hogweed (*Heracleum mantegazzianum*). Ragweed is an annual weed now spreading quickly, infesting at least eight Eurasian countries. The species represents a peculiar case because it is a serious issue for human health with pollen causing severe allergies. Silverleaf nightshade foliage and unripe fruits contain dangerous levels of solanine, while mature berries contain also high levels of asteroid alkaloids such as solanine and solanosine, which are toxic to livestock. Animals should be removed from infested areas until control is achieved. Direct contact with hogweed leaves and/or stems may cause severe blisters on human skin, when glucoside phototoxins in the plants are activated. This review reports lists of natural enemies known for each species and their known use in biological control programs outside of Eurasia.

# Using augmentative biocontrol against *Euphorbia esula*: an innovative program in France

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Native from Eurasia, *Euphorbia esula* ssp. *esula*, leafy spurge, is usually found in grasslands and disturbed areas in France. In the flooded meadows of the Saône Valley (Ain, Saône-et-Loire), an unusual spread has been observed since the 1990s affecting the management of grasslands. The increase of this *E. esula* could be a threat for this ecosystem of European importance (Natura 2000 site), and for plant and bird diversity. Chemical control, commonly used by farmers since the end of the 1990s, remained uneffective as previously observed in the US. In that regard, since 2003, we made extensive surveys for collecting indigenous natural enemies in leafy spurge stands. Seven indigenous natural enemies, including a rust, were observed on *E. esula*, of which the cerambicyd beetle, *Oberea erythrocephala*, and the cecidomyid fly, *Spurgia* sp. were particularly studied. Natural infestations of *O. erythrocephala* larval stage ranged 11 to 26% in 2006. Attempts to rear both species are described, particularly with the beetle on an artificial medium. In Spring 2006, 100 field-collected and artificially reared adults were released on a 10-sq.m. plot naturally covered with leafy spurge. Damage and infestation rates were compared to controls. In addition, choice and no-choice tests were estimated with *E. esula* and *E. palustris*, a local protected native plant. Considerations about the future use of *O. erythrocephala* for augmentative release is discussed.

# The biological control of *Impatiens glandulifera* Royle

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*Impatiens glandulifera* Royle is a highly invasive weed which has successfully invaded almost every riparian system in the UK after its introduction from the Himalayas as a garden ornamental in 1839. Now one of the tallest annual plants in Europe, *I.glandulifera* is able to outcompete native species due to its vigorous growth rates, large seed banks and prolific seed dispersal. When *I. glandulifera* forms monocultures in riparian habitats, the effects on the ecosystem can be severe, potentially causing bank erosion, biodiversity loss and increased risk of flooding. The environment agency estimates it would cost between £150–£300 million to eradicate the plant in the UK. In August 2006 CABI scientists conducted a survey of the natural enemies of *I. glandulifera* in its native range (foothills of the Himalayas, Pakistan). High levels of damage caused by arthropods and fungal pathogens were observed in all populations sampled, and the most interesting agents were collected and shipped back to the UK for further testing. This paper presents the results the work conducted in 2006 and explores further the potential for developing this work into a full biocontrol program against *I. glandulifera* in Europe.

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## Theme 8:

# Release Activities and Post-release Evaluations

## Session Chair: Rosemarie De Clerck-Floate

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Keynote Presenter

# Release strategies in weed biocontrol: how well are we doing and is there room for improvement?

**S.V. Fowler,[1] H.M. Harman,[2] J. Memmott,[3] P.G. Peterson[4] and L. Smith[1]**

*Summary*

A large number of factors associated with the agent and habitat can be manipulated when making a biocontrol release. Yet, it is only recently that an experimental approach has been taken, particularly with the release of arthropod weed biocontrol agents, for example examining factors such as release size to test ideas originating from theoretical or retrospective studies. For release size, both retrospective analyses and experimental studies with arthropod agents usually show that larger releases have a higher probability of establishing. However, a limited number of large releases at a few sites also run the risk of chance extinction from locally major environmental effects. These risks can be minimized by releasing at many widespread sites, so there is often a trade-off between a few large releases and many smaller one. Unless these risks and relationships are known, a mixed strategy with arthropods, using a range of release sizes, is likely to be optimal at least at the start of a release programme. Recent theoretical attention has been applied to specific mechanisms creating lower individual fitness in small populations, termed Allee effects. It appears that Allee effects, including genetic inbreeding and problems in finding mates, may have been underestimated: They can be powerful, as witnessed by deliberate extinctions using sterile male release. Genetic issue with biocontrol releases, such as strain selection, inbreeding depression, drift and creation of laboratory-selected strains, have also received considerable, mostly theoretical, analysis. However, the actual rate of establishment success per agent species in weed biocontrol programmes is now 80–100%. These overall analyses do hide problems in establishing particular agent species: Programmes in New Zealand targeting 'recalcitrant' environmental weeds have been hampered by partial or complete failure to get potentially critical agent species established. A case study of heather beetle in New Zealand is used to illustrate the frustrations and research challenges in a release programme.

***Keywords***: release size, Allee effects, genetics of biocontrol releases, establishment rates, heather beetle.

## Introduction

Classical biological control offers just about the only opportunity to make multiple experimental releases of an exotic organism into a new environment. Despite this, most studies of the effect of different release strategies until recently relied on retrospective analyses (Hall and Ehrler, 1979; Beirne, 1985; Simberloff, 1989; Hopper and Roush, 1993). Many of these studies called for a more rigorous experimental approach in classical biological control programmes, and recently, this call has been heeded (Memmott *et al.*, 1998; Grevstad, 1999a; Memmott *et al.*, 2005), although there are usually constraints in operational programmes because of the additional expense of experimental releases (McFadyen, 1998). This paper reviews developments with release strategies, for example research on the effect of release size on establishment success. Inevitably, our review is biased towards arthropods used for weed biocontrol, largely because more research has been done regarding

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release strategies with arthropod agents. However, many of the issues also affect pathogen agents, and we highlight areas where pathogen release strategies have received research attention. We also ask what the establishment rate is for new agent releases in well-resourced biocontrol programmes and whether there is room for improvement. Lastly, we use the release of heather beetle in New Zealand to illustrate some of the ecologically interesting challenges that a partially successful release programme can face.

## How to make releases of weed biological control agents?

In biological control releases, a multitude of factors can be varied, some of which may have a dramatic effect on the probability of the agent establishing a self-perpetuating population (Day *et al.*, 2004). Factors that can be manipulated in biocontrol release programmes can broadly be divided into the following:

1. Agent stage/quality/release timing: Releases can often be made at a variety of seasons, using a variety of life history stages of an agent (e.g. eggs, larvae, pupae and adults for insects, various spore types or hyphal suspensions for pathogens and releases using entire plants for pathogens or insects). The number of individuals, and their genetic make-up, is obviously open to manipulation, but these areas have received sufficient research attention to warrant their own section below.
2. Habitat/environmental quality: Release sites are selected in biocontrol 'invasions', and apart from the requirement for the host weed to be present, many other factors may be important (including some form of climate match if the weed occupies a wider range of climatic zones than are optimal for the agent). Releases may be made initially into cages or other protected environments. This may be to protect low initial release numbers from abiotic factors, such as bad weather events, from biotic factors, such as predators or competitors, or simply to prevent unwanted dispersal of mobile stages. Other mechanisms to discourage dispersal might involve supplemental food for agents or treating the target plants with fertilizer to improve the habitat.

Given the ease with which many of these factors could be explored experimentally, it is surprising that so little practical science has been done. However, recent studies have experimentally varied release size (see section below), and in other cases, the effect of simulated rain (Norris *et al.*, 2002) or rain protection (Hill *et al.*, 1993) on releases has been quantified. There are also examples where different release strategies for pathogens have been compared (e.g. Sheppard *et al.*, 1993). Recent research, including quantitative field experimentation, under the CRC for Weed Management in Australia has been reviewed by Day *et al.* (2004).

Small populations are perceived as vulnerable to poor performance that may result in extinction. If this vulnerability is not a feature of large populations, then this is often termed an Allee effect, after this issue was first raised by Allee *et al.* (1949). Allee effects could become particularly important if the population size drops because of say bad weather soon after the release. Allee effects could also cause, or be enhanced by, a released population that fails to grow rapidly because of poor individual performance. A corollary of this is that, for a biocontrol agent to be successful, any poor performance at low densities will need to be improved at higher densities. For example, if predation causes major problems for agent releases, then continued effort with that agent might only be worthwhile if this predation is suspected to be an Allee effect (and thus will decline as an issue once populations rise above a certain threshold size).

With any new agent species, there will be uncertainty over the optimal way to release. Consequently, mixed strategies are probably best at the early stages of a release programme. Ideally, the effect on establishment rates of various release strategies will be explored using rigorous experimental techniques (and/or adaptive management approaches), but political or funding realities may interfere (McFadyen, 1998). Uncertainty in optimal release strategies has been dealt with explicitly in modelling optimal release sizes (see below).

### Optimal release size

Retrospective analyses of arthropod agents show that establishment rates improve with increased size of releases (Hall and Ehler, 1979; Beirne, 1985; Simberloff, 1989; Hopper *et al.*, 1993), but even large releases can be exterminated by unusually severe, but local, stochastic environmental effects such as floods, droughts or inadvertent weed control. Therefore, there is a trade-off between making few large releases, and risking chance local extinction, and spreading this risk (over time and/or space) among a larger number of smaller releases (Grevstad, 1999b). However, small populations are also clearly at much more risk of extinction from chance events (both environmental and demographic stochasticity) than larger populations. Demographic stochasticity refers to chance changes in say birth and deaths, which are unlikely to cause extinction in anything other than very small populations. In contrast, Allee effects on small populations are considered to involve specific mechanisms reducing individual fitness at small population sizes. The most significant for biological control are likely to be genetic inbreeding and loss of heterozygosity in small populations leading to decreased individual fitness (see the next section) and problems in finding mates when a population is small. In general, the importance of Allee effects has only recently been recognized (Courchamp *et al.*, 1999; Stephens and Sutherland, 1999). Allee ef-

fects can be powerful, as demonstrated by deliberate extinctions of pest species by mass release of sterile males, which induces major Allee effects in relatively large populations (Courchamp *et al.*, 1999).

Simulation models (Grevstad, 1999b) suggest that demographic stochasticity alone is not likely to be important in the establishment of biocontrol agents, while Allee effects and environmental variability are crucial. In a variable environment, a large number of very small releases will maximize the chance of overall establishment because environmental variability reduces the likelihood of establishment over the entire range of colony sizes (Grevstad, 1999b). In contrast, when an Allee effect is present (in a constant environment), a single large release is optimal because the presence of an Allee effect results in a population establishment threshold. For colony sizes below the threshold, a population will become extinct, while those above the threshold will establish (Grevstad, 1999b).

Memmott *et al.* (1998) investigated the relationship between release size and the probability of establishment of gorse thrips, *Sericothrips staphylinus* Haliday, in New Zealand. A higher proportion of small releases became extinct: Thrips were recovered from 100% of releases of 270 and 810 thrips but from only 33% of releases of 10, 30 and 90 thrips. This suggested that the optimum release size for gorse thrips in New Zealand might be fewer than 100 (Memmott *et al.*, 1998), in contrast to the previous strategy of 1000 thrips per release.

In a 5-year experiment in New Zealand, different release sizes of the broom psyllid *Arytainilla spartiophila* (Förster), were monitored (Memmott *et al.*, 2005). Local extinction was greatest in the first year, and although the probability of extinction in the first year was related to release size, several releases of just one pair of psyllids resulted in established populations. Similar results were obtained by Grevstad (1999a) using two chrysomelid beetles in the United States. Thus, releases of even very small numbers can result in establishment, albeit with a higher probability of early extinction compared with releases of larger numbers of individuals.

These conflicts were further investigated using a modelling approach by Shea and Possingham (2000). They found that it was always possible to find one optimal release size that was better than any mixed release size strategy but only if the relationship between establishment rate and release size was known. Early in a release programme, it is better to use a range of release sizes to help determine the optimum. However, spatial variability in suitability of release sites, if unknown a priori, encourages a more risk-adverse release strategy (i.e. more small releases).

Overall, the issue is of inherent uncertainty, whether in defining an optimal release size or in the problems likely to be encountered with spatial variation between sites or just plain bad luck in the stochastic events that could cause local extinction of a release. Consequently, current release strategies for insects in New Zealand use a range of release sizes early in a programme. This maximizes efficiency while reducing the risk of complete failure in case there is a currently unknown threshold release size below which establishment is unlikely. As a result, a dataset of release size and establishment success across a range of species is slowly being accumulated.

## Genetics and releases of biological control agents

There have been several reviews on genetic issues concerning rearing/culturing and releasing biocontrol agents, concentrating mainly on arthropods (Mackauer, 1976; Roush, 1990; Hopper *et al.*, 1993; Hufbauer and Roderick, 2005). Genetic issues, which are acute in small populations, include the loss of alleles through genetic drift and inbreeding depression, especially where the mating of siblings increases the likelihood of homozygous deleterious recessive alleles. Another genetic issue, not related to population size, is the possible selection for laboratory-adapted populations, particularly when colonies are maintained for too long in artificial conditions.

The significance of inbreeding depression in field populations has been contentious, but in a landmark study, Nieminen *et al.* (2001) found that the egg-hatching rate in laboratory experiments with the butterfly, *Melitaea cinxia* (L.), was significantly lower in egg batches laid by inbred females compared to crossbred females. In field studies, using released inbred and crossbred populations, fewer inbred populations survived after 1 year (Nieminen *et al.*, 2001).

The only experimental test on how laboratory rearing affected a biocontrol agent that we could find used the geometrid moth, *Chiasmia assimilis* (Warren), a biocontrol agent against *Acacia nilotica* (L.) in Australia. In this study, Wardill *et al.* (2004) reared a range of isofemale lines from several sites. From generation 3, they mixed the lines and sites, creating several control lines to mimic the ‘bulk-rearing’ typical of biocontrol programmes. At generation 8, they created replicate-mixed isofemale lines within and across sites. Genetic variation was reduced in single isofemale lines in generation 8 compared with generation 1. However, the within- and across-site hybrid lines maintained high genetic variability. Fecundity in generations 8 to 11 showed a similar pattern. Thus, hybridizing isofemale lines appeared to restore genetic variation and fitness (Hopper at al., 1993). However, ‘bulk-rearing’ over four to six generations also maintained genetic variability and fitness, perhaps because of the minimum population size of 30 females and 30 males.

Overall, the significance of genetic issues in releasing biological control agents remains unresolved. Crucially, we have little evidence that releases fail to

establish, or do well, because of genetic factors such as strain/provenance selection, inbreeding depression or lack of adaptability. Experimental research during the release and establishment of biological control agents could offer considerable insights. In the meantime, it is probably prudent to follow some or all of the guidelines suggested by earlier comprehensive reviews (e.g. Hopper *et al.*, 1993) such as:

- Collect from a wide geographic range and match weed biotype and agent pathotype/provenance where known.
- Maintain separate lines in laboratory rearing (but release as hybrid mixtures).
- Rear for a limited number of generations in artificial conditions (e.g. no more than five).
- Keep rearing/culturing conditions similar to those in the field (e.g. fluctuating temperatures) to minimize adaptation to laboratory conditions.
- Avoid genetic bottlenecks or ensure that they are short (one generation) and then expand the population rapidly to maintain heterozygosity.

Collecting from a range of sites to maximize the genetic variability of agents has often been used with arthropod biocontrol agents, but care is needed to ensure that, for example, host range is consistent between source populations. In contrast, releases of pathogens for biocontrol of weeds have typically used material from single lines in part because of unjustified concerns over the potential evolution of expanded host ranges (Morin *et al.*, 2006b). As a result, the biocontrol programme against blackberry, *Rubus fruticosus* L., in Australia had, until recently, only used rust strains that were effective against a limited number of biotypes of the weed (Morin *et al.*, 2006a). Matching weed biotypes and agent pathotypes is clearly a vital issue with selection of pathogens as weed biocontrol agents (e.g., Morin *et al.*, 2006a,b). With both arthropod and pathogens agents, there is a need to show that the tactic of releasing genetically variable weed biocontrol agents to encourage adaptation (e.g. to additional biotypes of a target weed or to a wider climatic range) is not going to increase the risk of agents expanding their host range to include non-target plant species. Indeed, a range of studies outside biological control show that rapid evolutionary changes are possible (Orr and Smith, 1998; Thompson, 1998). However, the role of adaptation in the success of biological control agents remains unresolved and open to debate (Hufbauer and Roderick, 2005). From retrospective studies, we can say that expansion of the host range of weed biocontrol agents as a result of evolutionary adaptation does not seem to have happened (van Klinken and Edwards, 2002), and, for example, the substantial surveys to check for attack on non-target plants in New Zealand are showing that host-range testing procedures dating back to the late 1920s were generally very reliable (Paynter *et al.*, 2004; Landcare Research, unpublished data).

## What establishment rates are programmes achieving?

There are different ways of measuring establishment rates, each subject to biases. For example, the quoted establishment rates per weed biocontrol agent species of 71% (Julien *et al.*, 1984) is a simple and probably quite robust statistic, but it ignores whether establishment of an agent has failed at different sites or within countries. Rates per agent/country can also be calculated, with Julien *et al.* (1984) showing this to be 64%. This takes into account different experiences in different countries but again misses establishment failure in areas within countries. Rates of establishment *per release* appear to be seldom known, although the specific release size experiments discussed earlier are an exception to this. Syrett *et al.* (2000a) also report that establishment rates per release for nine agent releases across a range of pasture weeds in Australia varied from 12% to 92%. In New Zealand, it is apparent that the effort put into monitoring of any given agent can vary through time, adding variability to overall establishment rates per release. In addition, if some strains were better than the others or local adaptation was important, then differences in establishment rates might emerge over time within a biocontrol agent species.

In New Zealand, the overall establishment rate per species of weed biocontrol agent has increased from a reported 44% (Cameron *et al.*, 1993) to 76% (Fowler *et al.*, 2000; Syrett *et al.*, 2000a). Indeed, if the establishment rate is calculated for releases after the nationwide technology transfer programme was set up in the 1990s, then it increases to 95% (Hayes, 2000). Another region that has invested strongly in release and redistribution strategies is Oregon, USA, where the establishment rate per species is 81% (McEvoy and Coombs, 1999). If pathogens are considered separately, then the establishment rate is 100% for both New Zealand and Australia (Morin *et al.*, 2006b). On the basis of these statistics, it would seem that there is not a great deal of room for improvement in establishment rates for agent species for weed biocontrol in areas where substantial effort is invested into release, redistribution and monitoring. However, failure to establish any agent species that is released in large numbers can be frustrating. In New Zealand this has been the case with the sawfly, *Monophadnus spinolae* Klug., which is one of the few adequately host-specific agents available for the serious environmental weed *Clematis vitalba* L. The species establishment rate can also hide examples where establishment has been achieved but only at a poor rate per release: The next section discusses such a case example. Establishment per se is not the aim of weed biocontrol programmes, and it appears that despite high rates of agent establishment, the proportion of agents that appear to contribute to weed suppression remains stubbornly low. For example, McFadyen (1998) gives

agent establishment rates of 74% (in programmes that were rated as successful overall), but a proportion of agents established *and* contributing to control of only 55%. In New Zealand, establishment rates for all agents released exceed 80%, but of those established, we rate only around a third as contributing to weed suppression (Landcare Research, unpublished data). A similar pattern is emerging for pathogens, with 100% establishment in Australia, but only 33% of species contributing to weed control (Morin *et al.*, 2006b).

## A case study: heather beetle releases in New Zealand

Heather, *Calluna vulgaris* (L.), was deliberately introduced in Tongariro National Park in 1912. It now infests 50,000 ha, including one third of the park. Heather displaces native vegetation (Williams and Keys, 1994), and biological control is the only practical management option in the park. The biocontrol programme (Syrett *et al.*, 2000b) relied on just one agent species, the heather beetle *Lochmaea suturalis* (Thompson), because this beetle was such a well-studied species, which causes major damage to valued indigenous heather in Europe (Cameron *et al.*, 1944; Brunsting, 1982). In 1995, beetles were collected from a range of sites in the UK to ensure a climatic match to the national park (Emberson, 1986). A debilitating microsporidian disease was commonly found in heather beetles imported from the UK. To eliminate this disease, beetles in quarantine were reared from isofemale lines, with lines being destroyed if sampled offspring were diseased (Smith *et al.*, 1998). Lines were mixed before main set of 16 releases in the national park in Summer 1997/98, but two releases in summer 1995/96 were from one or two lines (second generation after field collection) each from the single geographic areas of Oakworth, England and Glencoe, Scotland. These first two releases were from lines that were doing particularly well, so beetles were available without disrupting the progress of the main rearing effort. All release sites were sampled intensively for beetle adults or larvae at 1- to 2-year intervals. No recoveries were made until summer 1999/2000, when five adult beetles and 20 larvae were found at Te Piripiri, a site that had received a covering of volcanic ash in June 1996, 5 months after the release. Despite intensive collecting efforts, no other recoveries have been made from any of these releases. Thus, our establishment rate from releases made from captive-reared heather beetle imported in 1995 is only 6% (Table 1).

The microsporidian pathogen was detected in one captive-rearing line in May 1999. All populations of heather beetle in captivity were then destroyed. At Te Piripiri, heather beetle numbers increased exponentially from 1999/2000 to 2001/2002 (Fig. 1), causing severe damage to heather. We urgently redistributed beetles from this localized, established population and also set up a secondary captive-rearing facility nearby. We trialled other release methods, including releasing larvae (in one case with a release size of 6000) and, in one case, used a much larger number of releases of just ten adults per release. We also made three releases in heather infestations near Rotorua, about 130 km north of Tongariro National Park, and at an altitude of 400 m (compared to 650–1300 m for sites in and around the national park).

Unfortunately, the releases in the national park using beetles collected from the one site where beetles were causing severe damage to heather suffered from a similar very low establishment rate as the original releases (Table 2). However, all three releases at Rotorua established and caused severe damage to heather within 2 years. Varying release parameters (±caging, early vs late summer, using larvae, release size) did not appear to improve establishment in the national park. After 2001/2002, the population numbers of beetles at the Te Piripiri declined markedly (Fig. 1). This collapse in numbers occurred over an area of about 1 ha and included outlying heather patches that were not noticeably damaged, so food shortage was not a viable explanation. The population of beetles was being carefully monitored, and this, together with a set of experimental studies, showed that predation, parasitism or diseases could not be implicated in the population collapse (Peterson *et al.*, 2004). Although we had done simple climate matching before collecting and releasing heather beetle, local weather conditions were the most likely explanation for both the collapse in numbers at Te Piripiri and the dramatically improved establishment rate at the lower altitude Rotorua sites. The spring weather in the year when the beetle populations at Te Piripiri collapsed was unusual, with what appeared to be a relatively warm end to the winter in September, followed by an exceptionally cold October/November with late snow. Nearby weather station data confirmed that October 2002 was the coldest since records began 18 years previously (Peterson *et al.*, 2004). We hypothesized

**Table 1.** The first releases of heather beetle in Tongariro National Park, using captive-reared beetles from the UK.

| Release season/year | Number of releases | Size of releases | Number of releases established |
|---|---|---|---|
| Summer 1995/96 | 2 | 250 | 1 |
| Summer 1997/98 | 13 | 100–800 | 0 |
| Summer 1998/99 | 2 | 250 | 0 |
| Totals | 17 | 5700 | 1 (6%) |

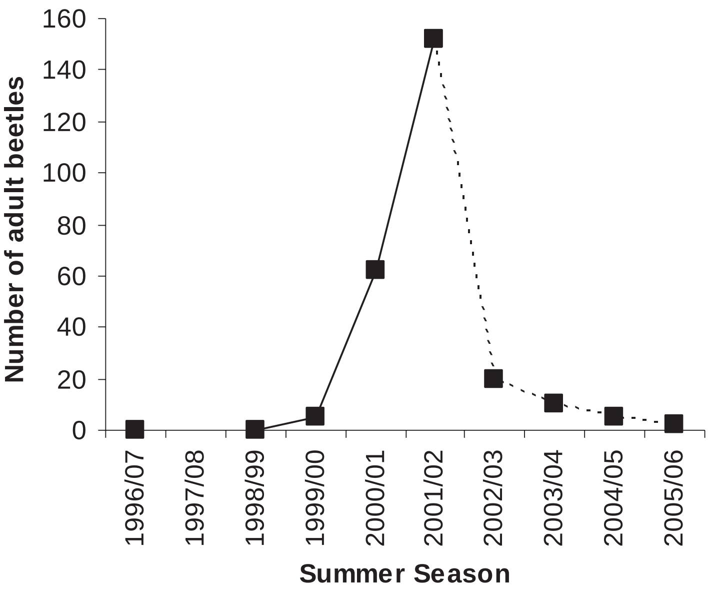


**Figure 1.** The numbers of adult heather beetles in a standardized sweep net sample at Te Piripiri. Numbers increased exponentially from the first detection in 1999/2000 until 2001/2002 but then crashed, apparently due to winter/spring weather conditions (further details in text).

**Table 2.** The second set of releases of heather beetle in New Zealand, using beetles redistributed and/or captive-reared from the first establishment site.

| Release region | Release season/year | Number of releases | Size of releases | Number of releases established |
|---|---|---|---|---|
| Tongariro | 2000/2001–2005/2006 | 49 | 10–6000 (total, 7500) | 2 (4%) |
| Rotorua | 2000/01 | 3 | 250 (total, 750) | 3 (100%) |

that beetles emerging from overwintering and their offspring would not be able to survive such major temperature fluctuations. In support, a Danish study reported that heather beetles had difficulty surviving winters with fluctuating temperatures, and individuals with smaller than average body size suffered particularly high mortality (Jensen and Nielsen, 1985). When we measured the body size of beetles collected from Tongariro National Park or Rotorua and compared them to field-collected beetles from a range of sites in the UK, to our surprise, we found that the New Zealand beetles were significantly smaller than those from the UK (Peterson *et al.*, 2007). Perhaps, small body size also increases beetle vulnerability to prolonged winters; furthermore, winters in Tongariro National Park are longer than those in Rotorua and Oakworth, England where surviving beetles were sourced from (Peterson *et al.*, 2007). Currently, we do not know whether this size difference is genetic or phenotypic. If genetic, then it is likely to be caused by genetic drift (founder effects) or inbreeding depression. A recent check of rearing records showed that the Te Piripiri release of 250 adult heather beetles was of second-generation beetles from just two isofemale lines, and one of these lines only contributed 29 beetles to the release. Unfortunately, we do not have preserved specimens of the original females or of their offspring in subsequent generations. If the size difference is phenotypic, then we have to find why. One hypothesis is that the leached volcanic soils typical of heather infestations in North Island, New Zealand, lead to nutritionally poorer heather for the beetle. Increased soil nitrogen (e.g. from air pollution) has been implicated as a factor causing heather beetle outbreaks in Europe, resulting in conversion of valued heather heathland into grassland (Heil and Diemont, 1983). As an experimental test of the importance of nitrogen, we have been releasing heather beetle into paired plots at Tongariro National Park with or without fertilizer application since Spring 2005. By summer 2006/2007, we had the second outbreak of heather beetle in Tongariro National Park, which so far has destroyed heather in an area of about 1 ha. It may be a coincidence, but this outbreak started at the first fertilized release site we set up. This outbreak has spread beyond the small fertilized patch, so there could a threshold effect whereby high beetle populations act to increase

host plant nutritional quality as suggested for in *Salvinia molesta* Mitchell biocontrol in the 1980s (Room and Thomas, 1985). It may also be significant that the original establishment site, Te Piripiri, was unique among all release sites in receiving a heavy fall of volcanic ash in the winter after the release, which might have produced a nutrient flush. It is now over 11 years since the first releases of heather beetle in New Zealand, and a multitude of research questions have emerged; plus, to date, we have only suppressed heather over a minute percentage of the infested area.

## Conclusions

Experimental biocontrol releases are being carried out in some cases, after a plethora of papers calling for this. With arthropod agents at least, this has resulted in some neat practical science to add to the range of retrospective analyses and theoretical models aimed at optimizing release strategies. Multiple strategies to reduce risk of getting something wrong are the norm with arthropod agents, and commonsense and modelling both suggest this is a sensible approach. However, establishment success rates per agent species are quite high, so in terms of getting species *established*, this research may not help much (although some failed establishments can still be very frustrating). The major 'log-jam' appears not to be establishment per se but getting agents that are effective at weed suppression. The key issues with release strategies (used in a broad sense to include provenance/strain and rearing/culturing) are those that might improve agent performance. These are probably mostly genetic factors, such as strain/provenance selection, avoiding inbreeding depression, allowing for sufficient genetic variability to allow for post-release selection (although its importance has yet to be demonstrated) and ensuring that laboratory adaptation does not impinge on agent performance. Individual release programmes will nearly always begin with uncertainty regarding the best way to make releases, and some of the scientific challenges are illustrated by the case study of heather beetle in New Zealand.

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# Feeding impacts of a leafy spurge biological control agent on a native plant, *Euphorbia robusta*

**J.L. Baker and N.A.P. Webber**[1]

*Summary*

The biological control agent, *Aphthona nigriscutis* (Foudras), has been established in Fremont County, WY, since 1992. Near one release site, a mixed stand of leafy spurge, *Euphorbia esula* (L.), and a native plant, *Euphorbia robusta* (Engelm.), was discovered in 1998. During July 1999, *A. nigriscutis* was observed feeding on both leafy spurge and *E. robusta*. Thirty-six *E. robusta* plants were located and staked within a 2-ha area, which had a visually estimated leafy spurge canopy of more than 50%. Eighty-eight percent of the *E. robusta* plants had feeding damage. By August 2001, the leafy spurge canopy had declined to 6%, and the *E. robusta* had increased to 450 plants with just 5.7% having feeding damage. In subsequent years, the data followed the same pattern; however, in 2007, no feeding damage was observed. At that time, leafy spurge groundcover was just 3%. For the 9-year period, leafy spurge canopy was inversely correlated to *E. robusta* density and positively correlated to *A. nigriscutis* feeding damage, suggesting that as leafy spurge density declined so did *A. nigriscutis* feeding on *E. robusta*.

***Keywords***: *Aphthona nigriscutis*, non-target feeding, *Euphorbia esula*.

## Introduction

Site 1, a parcel of land 5 km southwest of Lander, Fremont County, WY, has been infested with leafy spurge, *Euphorbia esula* (L.), for over 30 years. *Aphthona nigriscutis* (Foudras) was redistributed to this site in 1996 from locally established populations. While monitoring *A. nigriscutis* at site 1, we observed a small colony of a native spurge, *Euphorbia robusta* (Engelm.)

Early in the leafy spurge biological control effort, *E. robusta* had been identified as a species of interest because it is closely related to leafy spurge, both belonging to the subgenus *Esula*, is a perennial that could support the long life cycle of *Aphthona* beetles and is sympatric with leafy spurge in North America (Pemberton, 1985). In 1997 and 1998, we observed *E. robusta* plants with feeding scars on the leaves and occasionally saw *A. nigriscutis* feeding on the plants. A few of those plants were marked for future monitoring, and the next year, the marked plants were gone. This feeding activity by *A. nigriscutis* could have been anticipated. An examination of the petitions to introduce *Aphthona flava* (Pemberton and Rees, 1990), *Aphthona cyparissiae* (Pemberton, 1986) and *Aphthona czwalinae* (Pemberton, 1987) in the United States showed that acceptance of *E. robusta* was almost as high as for leafy spurge. *E. robusta* was not used for host testing *A. nigriscutis* (Pemberton, 1989).

Early host-plant testing was designed to demonstrate that new biological control of weed agents would not attack economically valuable crop species. In recent years, testing also encompasses the impacts that biological agents might cause to native species. *Rhinocyllus conicus* (Frölich), a biological control agent for musk thistle, *Carduus nutans* (L.), has been found to impact a wide variety of native thistles, some endangered (Gassmann and Louda, 2001). Increased concern has stimulated a call for greater scrutiny of new biological control agents, more thorough study of the target species before release and post release tracking of host range under field conditions (Waage, 2001). It is in the spirit of post-release evaluation that these data are offered.

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## Materials and methods

Between May and August of 1999, site 1 was visited several times, and *E. robusta* plants were located, marked and photographed. The soils there are red loam, 50 to 150 cm deep. The site slopes 10° to 20° to the northeast. Average annual precipitation is 33 cm, although over the last 5 years, rainfall has been 50% to 75% of the normal rate.

The 2-ha study site included 36 *E. robusta* plants whose latitude and longitude had been recorded, and each was numbered using a wooden stake. Leafy spurge ground cover was estimated in 1999 and 2000. In 2000, the leafy spurge density was determined by taking 36 samples across the site using a randomly placed metre-square frame. In 2001, a permanent grid was established across the site at 15-m intervals, and ground cover for leafy spurge, *E. robusta*, annual and perennial grass and forbs, trash and bare ground were sampled annually at each grid intersection using a point frame and recording only the first contact with each pin (Levy and Madden, 1933). Plant density was determined after centring a metre-square frame over each grid intersection. Sampling dates were timed to capture maximum vegetation growth in late July to late August. Each marked *E. robusta* plant was revisited several times each summer from 2000 to 2007 and examined for feeding damage. Newly discovered *E. robusta* plants were marked with numbered wooden stakes, and the latitude and longitude were recorded and added to the list of plants to monitor.

Leafy spurge and *E. robusta* plants were dug and roots inspected for larvae and larval feeding damage. Ten leafy spurge and two *E. robusta* plants from near the study site were inspected in fall of 2000, and 12 plants per species from inside the site boundaries were inspected in 2001. In 2003, 30 plants per species were collected, with soil intact and held in cages, in a glasshouse and later inspected for emerging adults, larval presence and root damage.

For comparison with site 1, a second population of *E. robusta* was selected far from either leafy spurge infestations or *A. nigriscutis* releases. At this second site, located 33 km southeast of Lander, WY, *E. robusta* plants were marked and counted in 2000, 2001 and 2007.

## Results

Ground cover by plant class has been relatively constant since 2001. Leafy spurge ground cover fell from 50% in 1999 to 10% in 2000, 6% in 2001, and 6%, 9%, 9%, 5%, 2% and 3% through to 2007, respectively. Leafy spurge density did not show the same declining trend as leafy spurge groundcover (Table 1). This was explained by the reduced size in the individual plants. In 1999, the leafy spurge was 25 to 50 cm tall and heavily branched, while in later years, plants are mostly less than 20 cm tall, single stemmed and non-flowering.

The *E. robusta* population increased 15-fold by 2002. The 36 plants marked in 1999 at site 1 increased to 194 in 2000, 479 in 2001 and 542 in 2002. Thereafter, plant numbers declined to 411 in 2003, 456, 441, 391 and finally 307 in 2007 (Fig. 1). More than 600 plants were marked and evaluated over the study period 1999 to 2007. Of the original 36 plants, 15 (44%) remained to 2007, although two were dug up for evaluation. Three of the original 36 plants that were missing in 2000 reappeared in later years. In contrast, the *E. robusta* population at site 2 comprised 81 plants in 2000, 101 in 2001 and 76 in 2007. Of the original 81 plants, 54% or 67% survived in 2007.

Adult-feeding damage to *E. robusta* by *A. nigriscutis* declined on a percentage basis through the years from 87% in 1999, 48% in 2000, 5% in 2001, 2.6% in 2002 and then to 0%, except in 2006 when 1% (four plants)

**Table 1.** Plant density of leafy spurge, *Euphorbia esula* and *Euphorbia robusta*, and ground cover by plant class and species at site 1.

| Year | Density | | Percent Ground Cover | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leafy spurge (per m$^2$) | *Euphorbia robusta* (per m$^2$) | Perennial grass | Shrub | Perennial forb | Annual grass | Annual forb | Trash | Bare ground | Leafy spurge | *Euphorbia robusta* |
| 1999[a] | | | | | | | | | | 50% | |
| 2000[a,b] | 11.9 | | | | | | | | | 10% | |
| 2001 | 7.0 | 0.05 | 51.0% | 5.0% | 2.0% | 1.0% | 0.0% | 14.0% | 21.0% | 6.0% | 0.0% |
| 2002 | 9.2 | 0.07 | 45.4% | 8.2% | 2.5% | 0.7% | 0.0% | 17.9% | 19.6% | 5.7% | 0.0% |
| 2003 | 13.8 | 1.50 | 46.1% | 7.1% | 2.7% | 0.9% | 1.4% | 8.2% | 25.0% | 8.6% | 0.0% |
| 2004 | 24.8 | 1.50 | 45.7% | 8.9% | 2.7% | 0.0% | 1.4% | 12.5% | 20.2% | 8.8% | 0.0% |
| 2005 | 10.4 | 0.09 | 51.3% | 8.9% | 2.5% | 0.4% | 3.2% | 6.4% | 22.3% | 4.8% | 0.0% |
| 2006 | 6.3 | 0.09 | 48.6% | 7.3% | 1.6% | 0.4% | 0.0% | 16.8% | 24.0% | 1.8% | 0.0% |
| 2007 | 7.0 | 0.04 | 38.2% | 12.1% | 3.9% | 1.8% | 3.0% | 17.1% | 21.0% | 2.9% | 0.0% |

[a] Ground cover visually estimated.
[b] Density determined by randomly placed m$^2$ frame.

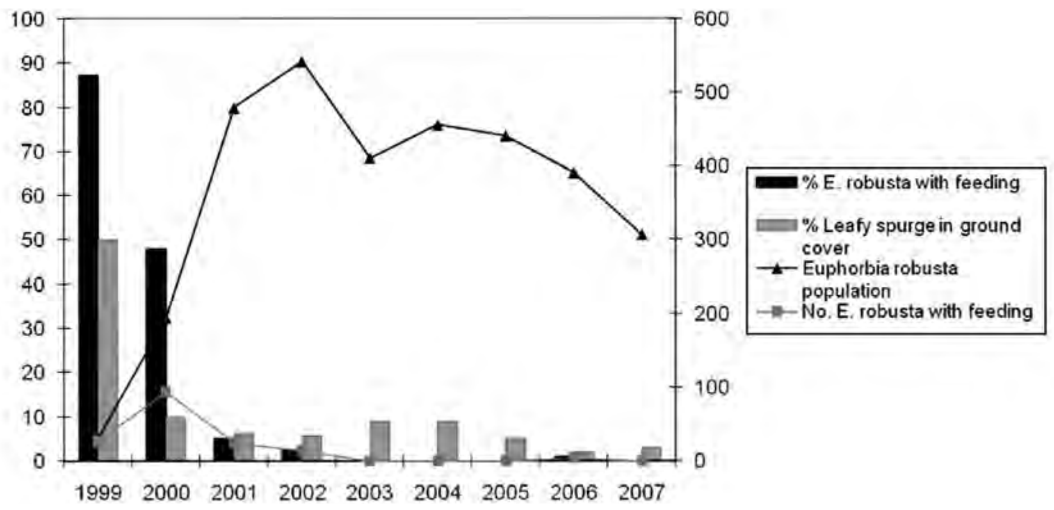


**Figure 1.** The changes over time for leafy spurge ground cover (%), *Euphorbia robusta* population size, feeding incidence (%) and number of *E. robusta* plants with feeding.

had minor feeding. An increase in actual numbers of plants with feeding temporarily followed the increase in total plant numbers but essentially fell to zero after 2002 with 2006 being the only exception (Fig. 1).

No larval feeding by *A. nigriscutis* on *E. robusta* was observed. In the fall of 2000, larvae could be found on the roots of three out of ten leafy spurge plants dug up at site 1. None were found on two *E. robusta* plants dug near this study site. In 2001, ten more plants for each species were dug up. Two of the *E. robusta* were plants with recorded adult feeding that year. No larvae were found on either species. In 2003, of the 30 plants of each species that were removed to the glasshouse with soil intact, only the leafy spurge produced any *A. nigriscutis* (Wacker and Butler, 2006).

## Discussion

As the *E. robusta* population increased over time, the number of plants with feeding damage decreased in numbers and percentages (Fig. 1). It appears that *E. robusta* was competitively suppressed by leafy spurge because, over the same period while the leafy spurge groundcover declined, the *E. robusta* population increased.

The feeding damage on *E. robusta* lagged a year behind the groundcover decline, suggesting that the adult feeding by *A. nigriscutis* in 2000 was more closely related to the leafy spurge ground cover in 1999 than in 2000 (Figure 1). The population at site 2 remained relatively constant over the same period with a similar turn over in plants as site 1, suggesting that *E. robusta* is a short-lived perennial with regular death and recruitment. Only where there was a decline in competition from the leafy spurge was there an increase in population recruitment.

Even though host-specificity testing predicted that *E. robusta* should be a good host for the *Aphthona* beetles, with 80% to 100% feeding acceptance and completed reproduction in the laboratory (Pemberton, 1986, 1987, 1989), observations at site 1 indicate that *A. nigriscutis* only fed heavily on *E. robusta* when its primary host leafy spurge was plentiful and able to support the biological control agent in large numbers. Even with a 15-fold increase, *E. robusta* did not show up in the ground cover measurements nor did it fill the space vacated by leafy spurge (Table 1).

It is not known if *A. nigriscutis* can complete its life cycle on *E. robusta* in the field. However, the strong correlation between the decline in leafy spurge with the decline in beetle damage to *E. robusta* and the absence of *A. nigriscutis* larvae on the *E. robusta* roots suggests that we observed a transient adult-feeding effect. A comparison of root morphology could explain this. The leafy spurge root is smaller in diameter and has more root hairs close to the surface, which may provide a better food source for developing larvae than *E. robusta* (Wacker and Butler, 2006). If *E. robusta* was a good developmental host for the beetle, then it would have been unlikely for the adult feeding to decline and the density of the *E. robusta* plants to increase with leafy spurge decline (R.W. Pemberton, 2003, personal communication).

This is in keeping with observations made in 1998 and 2001 at Camel's Hump west of Medora, ND. In 1998, this site was heavily infested with leafy spurge, which supported populations of *A. nigriscutis* and *Aphthona lacertosa* (Rosenhauer). The insects were superabundant, and millions were collected for redistribution in just a few hours. Every blade of grass had notches in the leaves, and the insects could be observed feeding

on every plant species present. By 2001, leafy spurge was nearly gone. At that time, no *Aphthona* sp. was observed feeding activity on any species other than the few remaining leafy spurge plants. Waage (2001) reported two parallel occurrences where weed biological control agents attacked non-target species during the epidemic period of agent development when the host plants were abundant. A lace bug, *Teleonemia scrupulosa*, released against *Lantana camara* in sesame crops in Uganda attacked the crop at peak populations (Davies and Greathead, 1967), and a leaf beetle, *Zygogramma bicolorata*, released against *Parthenium hysterophorum*, attacked sunflowers in India during population explosions (Jayanth *et al.*, 1993). In both cases, a decline in host-plant numbers resulted in a decline in the biological control agent and the non-target feeding stopped (Davies and Greathead, 1967; Jayanth *et al.*, 1993).

The *Aphthona* beetles are proving to be excellent biological control agents that severely impact their target weed, leafy spurge, in the United States (Nowierski and Pemberton, 2002). Their reputation can only be enhanced by these recently observed modest transient effects on their most likely non-target host, *E. robusta*.

## Acknowledgements

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# Variation in the efficacy of a mycoherbicide and two synthetic herbicide alternatives

**G.W. Bourdôt, G.A. Hurrell and D.J. Saville[1]**

*Summary*

Field experiments testing the efficacy of plant pathogenic fungi as inundative biological control agents usually reveal a wide spatial and temporal variation in the response of the target weed population. Such variation is typically thought to be much greater than that of synthetic herbicides. This perception may impede the commercial development of these fungi as mycoherbicides. In this study, we subjected this notion to an objective test using data from a total of 75 independent field experiments that quantified the responses of dairy pasture populations of giant buttercup, *Ranunculus acris* L., in New Zealand to a novel mycoherbicide utilizing the plant pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary, or to two synthetic phenoxy herbicides, 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 4-chloro-2-methylphenoxybutyric acid (MCPB). After excluding extreme and/or unreliable response estimates (one from 29 MCPA estimates, one from 14 MCPB estimates, and six from 32 *S. sclerotiorum* estimates), the mean percent reduction (±standard deviation) of the *R. acris* was 64% (±23), 39% (±22) and 51% (±20) for MCPA, MCPB and the *S. sclerotiorum* mycoherbicide, respectively. While these data indicate that the MCPA is on average more effective than both MCPB and the mycoherbicide, they provide no support for the notion that these two synthetic herbicides are less variable in efficacy than the mycoherbicide.

***Keywords***: biocontrol, *Sclerotinia sclerotiorum*, *Ranunculus acris*, weed control.

## Introduction

Plant pathogenic fungi used as mycoherbicides typically result in spatially and temporally variable reductions in their target weed populations. This phenomenon is well illustrated by experiments with *Sclerotinia sclerotiorum* (Lib.) de Bary (Brosten and Sands, 1986; Hurrell *et al.*, 2001) and, in the case of this and of other pathogens, is an impediment to their commercial development as mycoherbicides. This is particularly so when synthetic herbicide alternatives are available in the market place that are perceived to be more reliably efficacious. This problem has characterized and delayed the development of *S. sclerotiorum* as a mycoherbicide in New Zealand.

In this paper, we explore how well our perception, that the synthetic herbicides marketed for the selective control of *Ranunculus acris* L. (giant buttercup) in dairy pastures are generally more reliably efficacious than applications of *S. sclerotiorum*, matches up to the empirical evidence. We do this by comparing the variability and mean response of field populations of the weed to the two phenoxy herbicides, 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 4-chloro-2-methylphenoxybutyric acid (MCPB), in published experiments, with the variability and mean response to a novel formulation of *S. sclerotiorum* in a recently completed series of experiments. The two phenoxy herbicides were chosen for the comparison because they are the only ones for which data from individual experiments have been published; average responses only have been published for the two other herbicides registered for use against *R. acris* (thifensulfuron and flumetsulam; Lamoureaux and Bourdôt, 2007).

## Methods and materials

### Response of *R. acris* to MCPA and MCPB in historical experiments

In this paper, we use the responses of *R. acris* to MCPA in 29 published experimental tests (Tuckett,

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1961; Popay *et al.*, 1984, 1989; Bourdôt and Hurrell, 1990; Butcher *et al.*, 1993; Sanders *et al.*, 1994; Harris and Husband, 1997) and to MCPB in 14 experimental tests (Thompson, 1983; Popay *et al.*, 1989; Bourdôt and Hurrell 1990; Harris and Husband 1997) as reported in a wider study of the effectiveness of herbicides against *R. acris* in dairy pastures in New Zealand (Lamoureaux and Bourdôt, 2007). The percentage reduction in the measured response variable (either number of plants or percent ground cover) was derived from the mean responses (across field replicates) given in tabular or graphical form by the authors. Mean responses per experiment were used, as we were interested in the variability between, not within, experiments. We restricted our attention to applications made by the authors during the time of year recommended on the product labels [August–November (late winter–late spring) for MCPA and September–December (early spring–early summer) for MCPB]. This gave 29 and 14 individual tests for MCPA and MCPB, respectively, with mean application rates of 1.2 kg ai/ha for MCPA and 1.5 kg ai/ha for MCPB and mean assessment times of 25 and 23 weeks, respectively, after application. The mean application rates are lower than the current mid-point rates on the labels, 1.875 and 2.310 kg ai/ha for MCPA and MCPB, respectively (Young, 2008), but are likely to approximate the rate typically applied to dairy pastures.

## Response of *R. acris* to a *S. sclerotiorum* mycoherbicide

A series of experiments was conducted during 2004 to 2005 in which the fungal pathogen, *S. sclerotiorum*, was applied to small plots in *R. acris*-infested dairy pastures using a precision granule spreader (with infested farms randomly selected using standard stratified survey methodology). Three formulations were compared, but we consider only the most effective of them here: 'NF361', a dry polymer-coated flake containing mycelium adhering to spent barley (brewers' waste). There were 33 sites in total, 23 in the Golden Bay region and ten in the Taranaki region. Half of the sites were treated in early November 2004 (12 and 5 in Golden Bay and Taranaki, respectively) and half in mid-late November 2004 (11 and 5 in Golden Bay and Taranaki, respectively). There were two non-treated control plots at each site. The percentage of the pasture surface covered by *R. acris* was measured on each of three occasions (8, 14 and 20 weeks after application) using laser-assisted point analysis. In this paper, we report only the data recorded 20 weeks after application for a more valid comparison with the synthetic herbicides. The data for this assessment was missing for one of the sites where the farmer had sprayed the paddock with glyphosate before the assessment. For each of the remaining 32 sites, the percentage reduction in cover of *R. acris* due to the mycoherbicide was calculated using a 'Before–After, Control–Impact' calculation (Green, 1979). In this way, the impact of the mycoherbicide was assessed by comparing the changes in cover on treated and control plots between the assessments before and after treatment impact. The percentage reduction

$$100 - [(A_t/B_t)/(A_c/B_c) \times 100]$$

was calculated for each site. Here, *B* and *A* are *R. acris* groundcover at the time of treatment (before the impact) and after treatment (after impact), respectively. Subscripts t and c denote treated and control plots, respectively. This improvement in calculating the weed's response was not possible in the case of the historical data for MCPA and MCPB because the 'before impact' values were typically not reported.

## Statistical analysis

The mean percent reduction in *R. acris*, standard deviation and 95% confidence interval for the mean were calculated for each of MCPA, MCPB and the mycoherbicide. In addition, normal distribution curves were fitted to the means and standard deviations to illustrate the variation in the effects of the three herbicides. The three mean percent reductions were statistically compared using analysis of variance, and the standard deviations were compared using an *F* test.

# Results and discussion

Histograms of the percentage reductions of *R. acris* populations caused by the synthetic herbicides, MCPA and MCPB, and the mycoherbicide utilizing *S. sclerotiorum* are given in Fig. 1. The median percentage reduction for MCPA of 69.0 was substantially higher than for both MCPB and *S. sclerotiorum* (39.4 and 45.2, respectively; Table 1).

An alternative, more traditional statistical analysis of these data, based on the normal distribution, is problematical because of the occurrence of several extreme negative values. In one experiment involving MCPA and MCPB (Bourdôt and Hurrell, 1990), the control plot mean groundcover was exceptionally low, and this resulted in negative percentage reductions of −55% and −177%, respectively (Fig. 1). We omitted these values from the statistical analysis. Another problem was that, at four of the 32 sites involving *S. sclerotiorum* (two in each of Taranaki and Takaka), the initial cover of *R. acris* was very low (<6%) on the treated plot, resulting in unreliable estimates of the percent reduction (−367%, −314%, 45% and 100%). These data were also omitted from the statistical analysis. In addition, we omitted extreme values from two other sites involving *S. sclerotiorum* (−61% and −70%). In summary, we omitted all six negative responses and two positive responses.

It is interesting to note that, out of the total number of 75 experiments, we alone reported negative estimates (6 out of a total of 52); no negative estimates were reported by the other authors (in 23 experiments).

**Table 1.** Percentage reductions in groundcover of *Ranunculus acris* in dairy pastures in New Zealand resulting from applications of either of two phenoxy herbicides, MCPA (at an average of 1.2 kg/ha) or MCPB (average of 1.5 kg/ha) or a novel *Sclerotinia sclerotiorum*-based mycoherbicide (at 100 kg/ha).

| | Sample size | Median[a] | Statistical analysis using reduced samples[b] | | | |
|---|---|---|---|---|---|---|
| | | | Reduced sample size | Mean | Standard deviation | 95% confidence limits for the mean |
| MCPA | 29 | 69.0 | 28 | 63.6 | 23.3 | 63.6 ± 9.0 |
| MCPB | 14 | 39.4 | 13 | 38.7 | 21.9 | 38.7 ± 13.2 |
| *Sclerotinia sclerotiorum* | 32 | 45.2 | 26 | 51.0 | 19.8 | 51.0 ± 8.0 |

[a] Medians were calculated using the full sample.

[b] Sites were excluded from the statistical analysis if the initial cover of *R. acris* was very low (<6%) on either the treated or control plots, as these sites yielded unreliable estimates of the percent reduction. One such site involved MCPA and MCPB, and four sites involved *S. sclerotiorum*. Two other sites (involving *S. sclerotiorum*) giving unusually low estimates were also excluded.

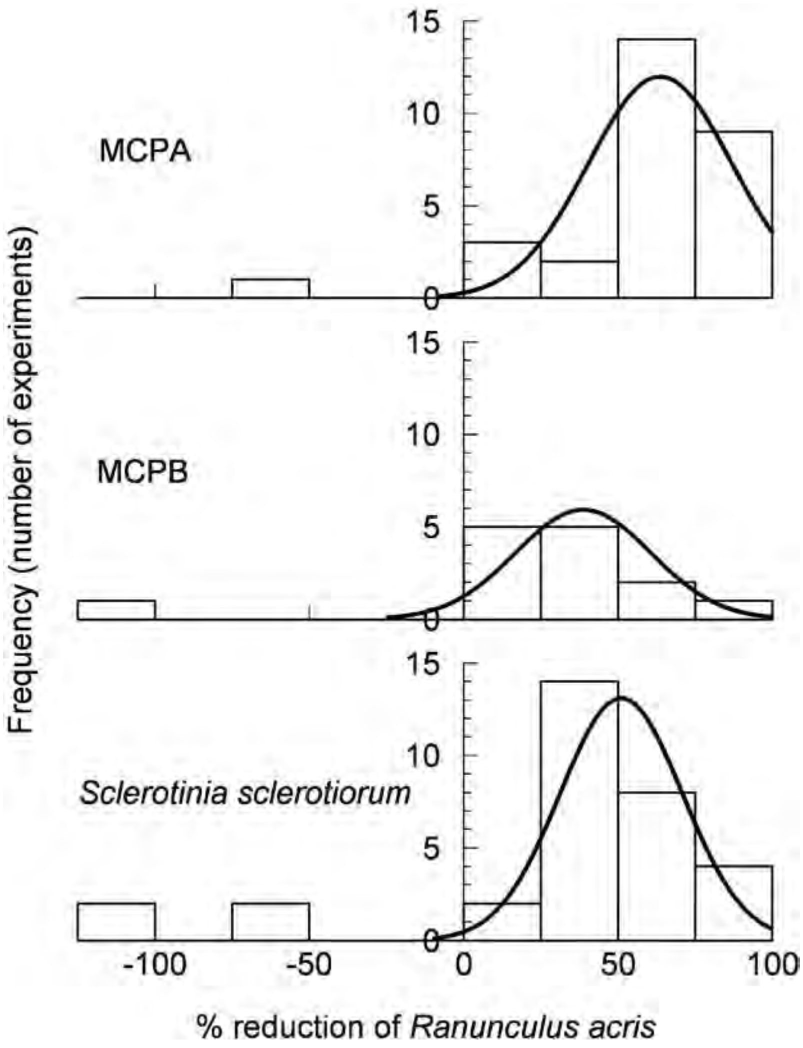


**Figure 1.** Histograms of the percentage reductions of *Ranunculus acris* populations caused by the synthetic herbicides MCPA and MCPB and the mycoherbicide utilizing *Sclerotinia sclerotiorum* (using size classes of 25% reduction). For MCPA and MCPB, these histograms display the data summarized in Table 1 of Lamoureaux and Bourdôt (2007). For *S. sclerotiorum*, the data have not been reported elsewhere. Note that the bottom class includes all values that are less than -100. Also displayed are normal curves using the means and standard deviations (based on reduced samples) given in Table 1.

We hypothesize that this is an example of the well-known phenomenon of 'publication bias' (Rothstein *et al.*, 2005), with negative estimates less likely to be published. In the present paper, our own research yielded all of the *S. sclerotiorum* estimates, 10 out of 29 MCPA estimates and 10 out of 14 MCPB estimates. As it seems likely that negative estimates for MCPA and MCPB have occurred in field experiments but not been published, the MCPA and MCPB histograms in Fig. 1 probably convey an overly favourable picture of the efficacy of these herbicides, while the *S. sclerotiorum* estimates, having been fully reported, convey a more realistic picture. As a consequence, we consider that our omission of the two negative values, –61% and –70%, from the *S. sclerotiorum* data leads to a fairer comparison with the synthetic herbicides.

Using the reduced data sets, the mean percentage reduction for MCPA of 63.6 was again substantially and significantly higher than for MCPB (38.7, $P < 0.01$) and *S. sclerotiorum* (51.0, $P < 0.05$; see means and their 95% confidence limits in Table 1). This is perhaps why MCPA is considered by dairy farmers to be a better choice than MCPB for the control of *R. acris*. The mean percent reduction for *S. sclerotiorum* was greater than for MCPB, but the difference was not statistically significant.

Interestingly, and contrary to popular perception, the *S. sclerotiorum* mycoherbicide was no more variable in its efficacy than the two synthetic herbicides ($F = 1.38$, $P > 0.05$; also see the standard deviations in Table 1 and the normal curves in Fig. 1). However, overall, variability was high. The causes of this wide variation in results for all three treatments are not evident from the experiments. Possible causes are (1) variation in weather conditions after application, (2) errors in application rates, (3) variation between experiments in the level of herbicide resistance (Bourdôt *et al.*, 1990) and resistance to *S. sclerotiorum* (Pottinger, 2006) and (4) variation in plant regeneration (from rhizome buds) and seedling recruitment after spraying (Brown, 1993; W. Brown, 1993, unpublished results).

## Conclusions and outlook

In conclusion, the mycoherbicide, utilizing the fungus *S. sclerotiorum*, would, if commercially available, be almost as effective against *R. acris* in New Zealand dairy pastures as the synthetic herbicide, MCPA (51% cf. 64% reduction, Table 1). This conclusion rests upon the assumption that resistance in *R. acris* to MCPA has not changed in intensity or spatial extent in New Zealand since the experiments we have reviewed in this paper were conducted. Our comparison also showed that, contrary to common perception, MCPA and MCPB are not necessarily more reliable against *R. acris* than *S. sclerotiorum*. Further testing of these preliminary results could be conducted through experiments that include both MCPA (and other synthetic herbicides) and *S. sclerotiorum* treatments. Given the wide variation in response that can be expected from both synthetic and biological control options (Fig. 1) and considering that the drivers of these variations are probably different between the synthetic and biological control options, it would be necessary to repeat such comparative experiments in both time and space. Experiments to determine the causes of the variation could, furthermore, lead to improvements in the effective use of both the synthetic and biological control alternatives and help determine the specific environmental conditions and market niches, which best suit the alternative approaches.

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# Ten years after the release of the water hyacinth mirid *Eccritotarsus catarinensis* in South Africa: what have we learnt?

**J.A. Coetzee,[1,2] M.P. Hill[1] and M.J. Byrne[2]**

*Summary*

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms, is the worst aquatic weed in South Africa, and biological control offers the most sustainable control option. The mirid, *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), was released against water hyacinth in South Africa in 1996 and shown to be damaging to the plant and host-specific within the Pontederiacae. Feeding, oviposition and nymphal development were recorded on pickerelweed, *Pontederia cordata* L., an important aquatic plant in North America but a potential weed in South Africa. The release of this agent allowed us to test in the field that pickerelweed was not part of the mirid's realized host range. The agent subsequently established at 15 sites around South Africa, including those where climatic modeling had indicated that it would not due to low winter temperatures, calling into question the usefulness of climate-matching techniques in the absence of microclimate and behavioural data. Hypertrophic nutrient conditions also reduced the effectiveness of *E. catarinensis* due to rapid proliferation of the plant, but the mirid reduced both the vigour and competitive ability of water hyacinth in mesotrophic and eutrophic water. *E. catarinensis* is emerging as an effective agent in areas of medium to low-nutrient status with a warm climate and should be considered for release in other areas of the world, particularly Africa, where few Pontederiaceae occur. This programme shows the value of considering fundamental vs realized host ranges but suggests that more data are needed to increase confidence in climate compatibility predictions.

***Keywords***: host specificity, realized host range, climate-matching, post-release evaluation, agent impact.

## Introduction

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms, remains the world's worst aquatic weed, even though up to seven biological control agents have been released against it in at least 30 countries (Julien and Griffiths, 1998). The effects of these agents are spatially and temporally variable such that water hyacinth still causes problems in many regions, including South Africa (Hill and Olckers, 2000). One of the newer agents against water hyacinth is the mirid, *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), which was screened and released in South Africa in 1996 as a new natural enemy of water hyacinth (Hill *et al.*, 1999).

Hill *et al.* (1999) found that *E. catarinensis* had potential as a control agent of water hyacinth in South Africa due to its host specificity within the Pontederiaceae and because it has long-lived, mobile adults that are obviously damaging to the plant – the four nymphal instars and the adults feed gregariously, resulting in chlorosis and ultimately death of the leaves (Hill *et al.*, 1999). Since 1996, the mirid has been released at least 18 sites in South Africa (Hill *et al.*, 1999) and has established at 15.

In this paper, we review the results of the last 10 years of research since the mirid's release. We have conducted a range of laboratory and field experiments to (1) further evaluate the realized host range of the mirid, (2) determine its thermal physiology and potential distribution in South Africa and (3) assess the impact it is likely to have on water hyacinth. Based on these findings, we

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were able to predict what might occur in the field once it was released, and over time, we have been able to assess these predictions.

## Host specificity

Before the release of *E. catarinensis*, host-specificity trials demonstrated that pickerelweed, *Pontederia cordata* L., an important, native, littoral plant of waterways in the United States, may be at risk because feeding, oviposition and nymphal development were recorded on it in the laboratory (Hill *et al.*, 1999). This did not prevent the release of the mirid in South Africa, as pickerelweed is neither indigenous nor economically important.

Hill *et al.* (2000) predicted that the results of the laboratory host-specificity tests were indicative of an artificially expanded host range, and despite feeding on non-target pickerelweed under laboratory conditions, the mirid would have minimal, if any, non-target effects on this species in the field, where natural host-selection cues would prevail. Since *E. catarinensis* had already been released in South Africa, we were presented with an ideal opportunity to test its realized host range in the field. We first attempted to force the mirid to establish on pickerelweed plants in the absence of water hyacinth by sleeving them onto leaves to prevent their initial dispersal (Coetzee *et al.*, 2003). The mirids fed on the leaves and produced offspring within the sleeves. Once the sleeves were removed after 5 weeks, the pickerelweed was monitored for establishment of the mirid. Under field conditions, *E. catarinensis* did not sustain a population on pickerelweed in the absence of water hyacinth (Coetzee *et al.*, 2003).

We also conducted choice tests in the field by placing pickerelweed plants among water hyacinth plants in a heavily infested river that had a large, well-established population of mirids. Monitoring of the pickerelweed plants showed that, although feeding damage was evident, it was far less than on water hyacinth and was indicative of spillover feeding damage because of the high mirid population levels (Hill *et al.*, 2000).

Therefore, the prediction of Hill *et al.* (1999) that, under restricted laboratory conditions, pickerelweed was a more suitable host for *E. catarinensis* than under field conditions, was correct.

## Thermal physiology

In South Africa, where at least five biological control agents have been released, water hyacinth control is not as successful as that in tropical areas (Hill and Olckers, 2000), and it was assumed that low winter temperatures play a crucial role in the successful control of water hyacinth in South Africa. Many of the worst water hyacinth infestations in South Africa occur at high-altitude sites that are typified by cold winters (Hill and Olckers, 2000). At these high elevations, water hyacinth infestations are subject to frost and winter dieback. Biological control in these areas is not as successful as that in frost-free areas because, in colder areas, regrowth of water hyacinth occurs during spring, whereas the insect populations only reach significant levels during midsummer (Hill and Cilliers, 1999). This lag period may allow the plant populations to increase unchecked and could be responsible for the variable results achieved by water hyacinth biological control agents in these regions.

When biological control agents are released into a new country, they should ideally be species or strains from a climatically matched area (Williamson, 1996). We therefore investigated various aspects of the mirid's thermal physiology to determine whether it might be limited by cold winter temperatures in South Africa. We determined the critical thermal minimum (CTMin, a point short of death where locomotory impairment occurs, but from which recovery is possible) of *E. catarinensis* to be 1.2°C and the lower lethal limit ($LT_{50}$, the temperature at which 50% of the population dies) to be –3.5°C (Coetzee *et al.*, 2007a). Neither of these limits is particularly low, and they might prevent the mirid from establishing in areas that receive considerable winter frost.

Another method available for climate matching is degree-day modeling, which uses temperature and time to predict the number of generations that an insect can complete at a given locality. We calculated that the mirid's thermal constant K was 342-degree days, above a developmental threshold *t* of 10.2°C (Coetzee *et al.*, 2007a). These values were then used to calculate accumulated degree days according to the methods of Campbell *et al.* (1974) for 128 South African localities, using the equation:

$$K = \sum \left\{ \frac{(T_{max} + T_{min})}{2} - t \right\}$$

The mean annual degree days accumulated for each location was then calculated, which predicted the number of generations that *E. catarinensis* could complete at each locality. The number of generations that the mirid could complete during the winter months (April to August) was also calculated. The results of both of these calculations indicated that fewer generations were possible at high altitudes, as was expected (Coetzee *et al.*, 2007a).

From these data, we predicted that low winter temperatures will limit the establishment of *E. catarinensis* in the field. This explained the failure of mirid establishment at Delta Park in Johannesburg, a high-altitude site, where it has been released at least three times in early summer, established, but then has not persisted through the winter as a consequence of heavy frosts. However, our prediction could not explain why the mirid did establish at another high-altitude site on the Vaal River, which experiences similar climatic conditions as Delta Park. It is likely that the effects of microclimates

provided by an abundance of overhanging vegetation played a major role in providing thermal refuges for *E. catarinensis* at the Vaal River site, thereby allowing it to persist through the winter. Furthermore, the mirid's behaviour might allow it to escape extreme temperatures in the canopy of the plant by moving down towards the crown in cold weather. Therefore, the assumption that standard meteorological data can be used to represent the conditions actually experienced by the insects in the field is a generalization (McClay and Hughes, 1995; van Klinken, *et al.*, 2003), highlighting the fact that we cannot ignore the effects of microclimates and behaviour on establishment patterns of the mirid in South Africa.

## Impact

Another factor contributing to the variable results achieved by water hyacinth biological control agents in South Africa is the effect of eutrophication of bodies of water (Hill and Olckers, 2000). Water hyacinth proliferation is usually closely linked to increases in eutrophication in these systems (Hill, 1999), and as a result, the effect of feeding by biological control agents is often insufficient to retard water hyacinth growth (Hill and Cilliers, 1999).

A previous study investigated the effects of herbivory by the mirid on water hyacinth grown at high, medium and low nitrogen (N) and phosphorus (P) nutrient concentrations under laboratory conditions (Coetzee *et al.*, 2007b). The results showed that water-nutrient concentration affected plant growth parameters of water hyacinth significantly more than did herbivory by the mirid. At high-nutrient concentrations, leaf and daughter plant production were more than double than at low-nutrient concentrations, while stems were twice as long at high-nutrient concentrations compared to low concentrations. Chlorophyll content was also twice as high at high-nutrient concentrations as at low concentrations. Although herbivory by *E. catarinensis* did not have as great an effect on water hyacinth vigour as nutrient concentration, it significantly reduced the length of second petioles, chlorophyll content of water hyacinth leaves and the production of daughter plants (Coetzee *et al.*, 2007b). These results are important because water hyacinth populations increase rapidly by vegetative reproduction through the production of daughter plants (Edwards and Musil, 1975), so any reduction in daughter plant production will have negative consequences for the rate of spread of water hyacinth.

From these results, we predicted that the mirid would have the greatest impact on water hyacinth infestations under mesotrophic conditions. At Clairwood quarry in KwaZulu-Natal, a eutrophic site, *E. catarinensis* has had a major impact on the infestation and is responsible for clearing the weed. Initially, large, brown, circular patches appeared in the mat, which gradually began to sink as the plants started to die. Eventually, most of the water hyacinth sank, and the majority of the impoundment remains clear, with fringing populations of water hyacinth. Based on examination of the plants in the field, the mirid is having an impact on the plants, as the leaves are brown and clearly chlorotic.

## Discussion and conclusion

In the 10 years that this agent has been established in South Africa, it has been shown to have a wider distribution than was first predicted (Coetzee *et al.*, 2007a), and while prediction of the insect's thermal resilience was reasonably accurate, the thermal buffering of microclimates considerably underestimated the eventual distribution. Inability to include local microclimates is an inevitable failing of models, which use climate envelopes as a basis for predicting distributions (Sutherst, 2003), while presence of the weed is not necessarily a predictor of climatic suitability for the agent (van Klinken *et al.*, 2003).

Laboratory host-specificity trials can produce ambiguous results, as the differences between the fundamental/physiological and realized/ecological host ranges are difficult to determine (van Klinken, 2000). Undertaking field host-range studies in the region of origin of the weed can often resolve these ambiguities, but in most cases, only plants common to both countries can be tested (Olckers *et al.*, 2002). Pickerelweed's presence in South Africa allowed validation of laboratory host-specificity results in the field, including assessment of the mirid's suitability for release in the United States. Hill *et al.* (1999) predicted that at best pickerelweed would be an inferior host in comparison to water hyacinth. Field trials confirmed that the mirid would not establish on pickerelweed in the absence of water hyacinth, but where the two grow sympatrically, some spillover feeding is expected (Hill *et al.*, 2000; Coetzee *et al.*, 2003). However, this spillover feeding on pickerelweed has not been quantified and possibly should be, before the agent is considered for release in the United States. In this case, the laboratory results overestimated the potential impact on the non-target species.

*E. catarinensis* was expected to contribute to the control of water hyacinth (Hill *et al.*, 1999), but its overall impact was predicted to be subtle in comparison to the *Neochetina* spp. weevils that are the mainstay of water hyacinth biological control (Coetzee *et al.*, 2005) and likely to be negligible in highly eutrophic conditions (Coetzee *et al.*, 2007b). Five to six years after release, mirid populations were generally low and their impact slight. However, in the past 2 to 3 years, several outbreaks of the mirid have been seen, resulting in water hyacinth mats collapsing, even at eutrophic sites. It is uncertain if these high populations of the mirid will persist, and this aspect warrants further study.

Since its first introduction to South Africa in 1992, there have been ten scientific publications on the biology, host specificity and impact of *E. catarinensis* on water hyacinth. Several of the predictions made about the agent proved to be accurate, while some were underestimates and others overestimates. Despite this close examination, we are still unclear of the interaction this agent will have with the other agents released against water hyacinth in South Africa (e.g. Ajuonu *et al.*, 2007) and its long-term impact on water hyacinth populations. Selection of a good agent retains the elements of art, even as we improve the science (Hoelmer and Kirk, 2005).

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# Release and establishment of the Scotch broom seed beetle, *Bruchidius villosus*, in Oregon and Washington, USA

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*Summary*

We provide a preliminary report on the Scotch broom seed beetle, *Bruchidius villosus* (F.) (Coleoptera: Bruchidae). This beetle was first recorded as an accidental introduction to North America in 1918. Host-specificity tests were completed before the beetle was released as a classical biological control agent for Scotch broom, *Cytisus scoparius* L. (Fabaceae), in 1997 in the western USA. Beetles were collected in North Carolina and shipped to Oregon in 1998. More than 135 releases of the beetle have been made throughout western Oregon and Washington. Nursery sites have been established, and collection for redistribution began in 2003. The bruchid's initial establishment rate is higher in interior valleys than at cooler sites near the coast and in the lower Cascade Mountains. Seed-pod attack rates varied from 10% to 90% at release sites that were 3 years old or older. Seed destruction within pods varied from 20% to 80%, highest at older release sites. *B. villosus* may compliment the impact of the widely established Scotch broom seed weevil, *Exapion fuscirostre* (F.) (Coleoptera: Curculionidae). *B. villosus* populations were equal to or more abundant than the weevil at seven release sites in Oregon. At sites where the bruchids were established, they made up 37% of the seed-pod beetle population, indicating that they are able to compete with the weevil and increase their populations. At several release sites older than 5 years, bruchid populations have become equal to or more abundant than the weevil's.

***Keywords***: *Cytisus scoparius*, *Exapion fuscirostre*, post-release assessment, pre-dispersal seed predation, biological control.

## Introduction

Scotch broom, *Cytisus scoparius* (L.) Link, Fabaceae, is native to central and southern Europe. It is a nitrogen-fixing leguminous perennial shrub 1–3 m tall with showy yellow pea-like flowers. Each plant may produce thousands of seeds that are long-lived, up to 80 years in the soil (Bossard and Rejmanek, 1994). Scotch broom was introduced into North America as an ornamental plant, but it escaped cultivation and became naturalized. Without its natural enemies to keep it in check, Scotch broom flourished and spread, particularly in disturbed habitats (Andres *et al.*, 1967). It occurs from British Columbia south to California and along the eastern seaboard of the USA, with major infestations in North Carolina (Andres and Coombs, 1995; Redmon *et al.*, 2000; Coombs *et al.*, 2004). Scotch broom has spread throughout the Pacific Northwest, west of the Cascade Mountains (Andres and Coombs, 1995), and is considered noxious in California, Hawaii, Idaho, Oregon and Washington. Most infestations occur where the natural plant communities have been disrupted by logging, construction, overgrazing and rights of way. In Oregon, where the plant infests the western third of the state, an annual economic loss was estimated at $47 million (Radtke and Davis, 2000). Much of the loss was associated with competition affecting reforestation after logging and the cost of control.

Because Scotch broom is a serious invasive pest in several countries, including Australia, New Zealand, Canada and the United States, an international program

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has been established to explore the possibilities for classical biological control (Syrett *et al*., 1999). Biological control of Scotch broom in the United States began in 1960 with the release of the twig-mining moth, *Leucoptera spartifoliella* Huebner (Lepidoptera: Lyonetiidae) (Frick, 1964; Andres and Coombs, 1995). Upon additional investigation, the moth was found to occur in the Pacific Northwest before its intentional introduction (Andres and Coombs, 1995). The seed weevil, *Exapion fuscirostre* F. (Coleoptera: Apionidae), was released in 1964 in California (Andres *et al*., 1967), in 1983 in Oregon and about 1989 in Washington (Andres and Coombs, 1995; Coombs *et al*., 2004). Large-scale implementation programs for collection and redistribution of the seed weevil have been conducted by the Oregon Department of Agriculture (ODA) (Isaacson *et al*., 1995). The seed weevil is now widely distributed, occurring throughout the Pacific Northwest (Coombs and Piper, 2002; Coombs *et al*., 2004). ODA surveys showed that the degree of seed reduction by the weevil alone (20–60%) was insufficient for long-term control, based on studies by Rees and Paynter (1977). Numerous other insects are associated with Scotch broom in the United States, many of which are adventive introductions (Waloff, 1968).

To further reduce Scotch broom seed production, an additional seed-feeding insect was sought to compliment the impact of the weevil. The Scotch broom seed beetle, *Bruchidius villosus* F. (Coleoptera: Bruchidae), was accidentally introduced in the eastern USA during the early 1900s (Redmon *et al*., 2000). It was first reported in 1918 in Massachusetts and is abundant in North Carolina, where seed reduction has been measured at more than 80% (Redmon *et al*., 2000). The bruchid was introduced in Oregon in 1998 and in Washington in 1999 (Coombs *et al*., 2004). This was the first adventive natural enemy in the United States to undergo host-specificity testing according to the US Department of Agriculture's (USDA) protocol to become a sanctioned classical weed biological control agent.

## Biological control agent

The life history of *B. villosus*, as provided by Southgate (1963) and Andres *et al*., (1967), is a univoltine insect that overwinters as an adult and is native to western and central Europe. Adults emerge from the duff in the spring and feed on Scotch broom pollen. After mating, gravid females may oviposit 2–12 eggs on the outside of Scotch broom seed pods. Larvae hatch and tunnel into the pod and begin feeding on the seeds. Usually, each larva develops in a single seed, completing development in 15–30 days. Pupation lasts 10–20 days and occurs within the seed coat. Adults are dark grey and about 2–3 mm in length. Adult feeding has little impact on the plant. Other plant species attacked include Portuguese broom, *Cytisus striatus* (Hill) Rothm., Spanish broom, *Spartium junceum* L., and French broom, *Genista monspessulana* (L.) L. Johnson.

## Methods

After the appropriate USDA permits were obtained, adult *B. villosus* were collected in the spring of 1998 in North Carolina and shipped to Oregon and later to Washington. Three main habitat types (coastal, valley and mountain) were chosen as release habitats where Scotch broom occurs. Releases of 100–400 adults per release were made, and site data were recorded. Releases of adult beetles during the mating and oviposition periods were preferred over releasing teneral adults. Selected release sites in each of the three main habitat types were sampled to evaluate establishment 4 or more years after release and to estimate numbers of adult *B. villosus*. Site evaluations included the use of beating sheets one to three times per year to verify presence of adults and sampling of three to ten plants per site, with three samples per plant. Seed pods (25–100 per site) were harvested to determine presence of eggs, larvae, adult seed beetles and numbers of damaged seeds. *B. villosus* was determined to be established when adults were recovered 3 or more years after release. When collection at nursery sites exceeded 200 adults per hour sampled, surplus beetles were harvested for redistribution to other sites.

## Results and discussion

The first release of *B. villosus* as a classical biological control agent for Scotch broom in the United States was made in western Oregon in August 1998. Unfortunately, the release cage used was either vandalized or blown away by strong winds, and the beetle was never recovered. Numerous releases were made in other areas from 1999 to 2001. In 2003, an established population west of Salem, OR, supported surplus beetles for local redistribution (Table 1). We anticipate that major collection and redistribution projects will occur during the coming decade.

**Table 1.** Year, number of releases and total number of *Bruchidius villosus* (F.) released in Oregon. First collections for redistribution began in 2003.

| Year | Number of releases | Number released |
|---|---|---|
| 1998 | 2 | 280 |
| 1999 | 17 | 3580 |
| 2000 | 8 | 1600 |
| 2001 | 30 | 7550 |
| 2002 | 3 | 450 |
| 2003 | 1 | 100 |
| 2004 | 1 | 200 |
| 2005 | 7 | 1300 |
| 2006 | 14 | 3100 |
| Total | 83 | 18,160 |

In western Oregon, more than 83 releases of *B. villosus* have been made throughout the core area of Scotch broom infestations (Fig. 1) and at least 52 sites in Washington. We attempted to include a wide variety of habitats that were infested with Scotch broom, including coastal, inland valley and mountainous. We sampled for adult beetles at 21 of 57 sites that were 4 or more years old. In 2005, we were unable to find *B. villosus* at seven of the 21 sites where a beating sheet was used, and three of the 26 sites where ripe seed-pods were collected. *E. fuscirostre* was found at all but one site in each of these surveys. We suspect that, as populations of *B. villosus* become better adapted to local climates and conditions, the number of sites without the bruchid will decrease.

Our observations showed that *B. villosus* populations required at least 4 years before sites were amenable to collection for redistribution. The bruchid does not appear to aggregate at new release sites and thus are difficult to recover until populations build up at a given site for at least 3 years. We were unable to find release sites that were not already inhabited by *E. fuscirostre* at the time of releasing *B. villosus* because of the earlier intensive collection and redistribution program conducted by ODA (Fig. 1). We hope to conduct tests to determine the population potentials and impacts of both beetles alone and in combination. Of the 21 release sites monitored for adult beetles, the mean ratio of the bruchid to the weevil increased with time, and at three 6-year-old sites was 50% or greater. Our data show that, despite competition with *E. fuscirostre*, *B. villosus* should continue to spread and increase (Fig. 2).

It is not yet clear which insect in which habitat is the better competitor without long-term monitoring. Data from the seed-pod analysis (Fig. 3) show that, with respect to *E. fuscirostre*, the overall proportion of *B. villosus* regardless of habitat sampled remains about the same when years-since-release is not factored in. Our sample size is inadequate at this time to clearly identify the trends. However, when we looked at establishment rates as a function of general habitat region, it was evident that *B. villosus* had a higher rate of establishment at the inland sites in the Willamette Valley of western Oregon (Fig. 3). The valley climate is drier and warmer than the coastal and mountainous sites.

Seed-pod attack rates were highly variable, from 10% to 90% at release sites that were 3 years old or older. Seed destruction within pods varied from 20% to 80%, being highest at older release sites. Analysis of seed-pod attack rates and seed destruction will be analyzed in future work.

There may be inherent problems in our early analysis that compared active adults collected by beating plants to the number of adults sampled in the seed-pod col-

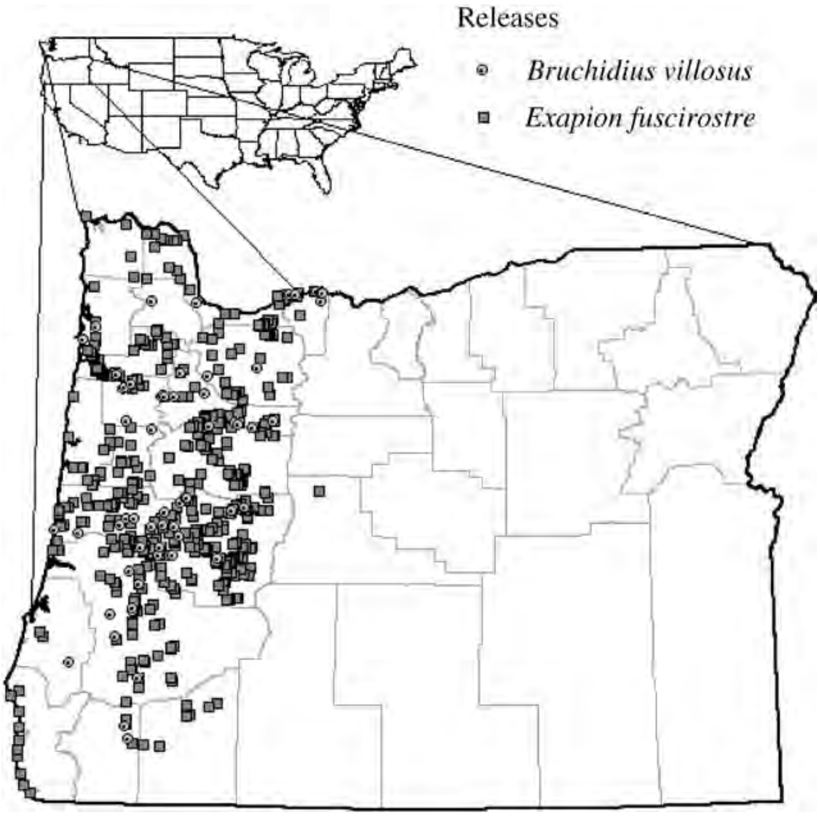


**Figure 1.** Release locations of *Bruchidius villosus* (F.) (circles; $n$ = 83) and *Exapion fuscirostre* (F.) (squares; $n$ = 529) on Scotch broom in Oregon, USA.

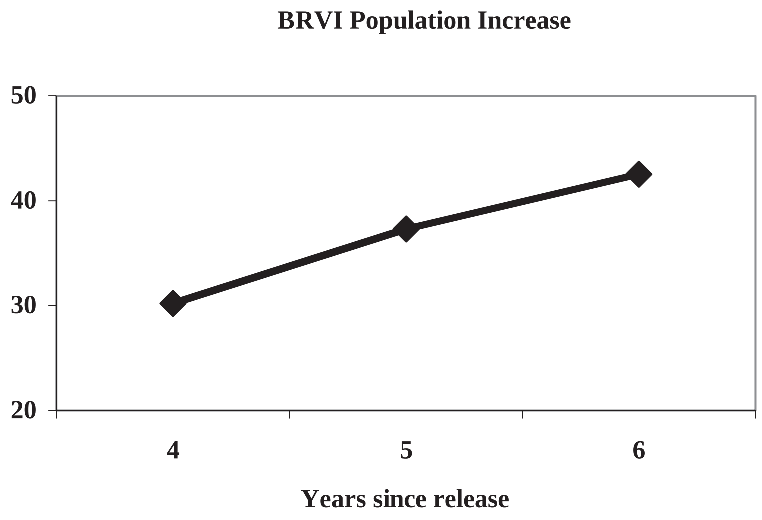


**Figure 2.** Mean increase of the 14 established *Bruchidius villosus* (F.) populations (from ripe seed-pod analysis) by years since release, as a percentage of the total seed-pod beetle population, including *Exapion fuscirostre* (F.), in western Oregon.

lections. Free-roaming adult beetles can move about; therefore, behaviour and abundance of adults of each species may differ in relation to plant phenology. This appears to be evident when comparing populations sampled on the same date from the cooler coastal and mountainous sites, which were phenologically behind those from the warmer and drier valley sites. Future analysis should be adjusted to specific degrees of plant phenology, e.g. percent in flower, average size or colour of pods. However, because of the variation that we have observed among and between sites, this may not be practical.

In the course of sampling the production of beetles in seed-pods, we found that a small percentage of beetle larvae were parasitized by chalcidoid wasps. Representative wasps were collected and sent to a USDA taxonomist for identification. The most common of the four species of pteromalids and the only one identified to species was *Pteromalus sequester* Walker, a natural enemy of *B. villosus* (Syrett *et al.*, 1999) and the gorse

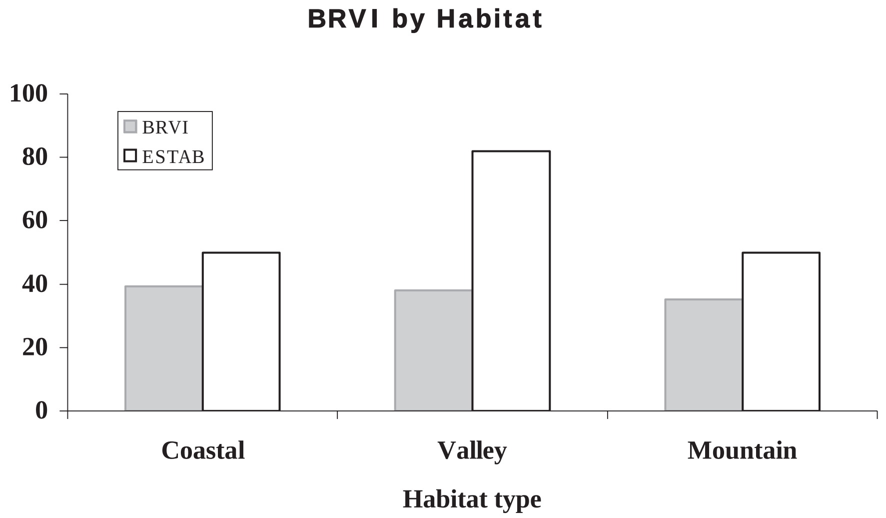


**Figure 3.** Percent of *Bruchidius villosus* (F.) releases, based on adult surveys, that were established (open bars) and the percentage of seed-pod beetle populations consisting of *B. villosus* (grey bars) by regional habitat type. The establishment rate of *B. villosus* was higher in the Willamette Valley region than in coastal or mountainous regions, but their population percentages in relation to *Exapion fuscirostre* (F.) were not.

seed weevil, *Exapion ulicis* Forster, in Europe (Davies, 1928). Our observations showed that parasitoid attacks on *B. villosus* and *E. fuscirostre* did not exceed 4% at any of the sites. We do not know how this parasitoid arrived in the United States, but we are concerned that they have the potential to render both seed-pod beetles ineffective as biocontrol agents.

Our studies show that *B. villosus* populations can establish and increase at the Scotch broom habitat types that we surveyed in the western USA. It is still too early to determine the degree of seed reduction that will be achieved by the combination of *B. villosus* and *E. fuscirostre*. Additional long-term studies are needed to document the spread and impact of both seed-pod beetles. As both species should become widely established, we hope that their impact will be additive, or at the very least, non-competitive. Based on studies by Rees and Paynter (1977), we expect that a 95% reduction of seed production will be necessary to achieve long-term control of Scotch broom infestations. At the present time, *B. villosus* appears to be better adapted to the warmer and drier climate of the interior western valleys of the American west coast. Additional studies are needed to determine the combined impacts of all natural enemies established on Scotch broom in the western USA.

## Acknowledgements

The authors wish to express their thanks to Dr. Timothy Forrest, University of North Carolina, Asheville, for providing the original releases of *B. villosus* for the western USA and to the many agency (ODA, BLM and USFS), county and university personnel who helped at various stages of this study. A special thanks is extended to Carol Horning, who spent many a long day making collections in the field.

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# Biological control of Mediterranean sage (*Salvia aethiopis*) in Oregon

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*Summary*

Mediterranean sage, *Salvia aethiopis* L., is a serious naturalized invasive plant of rangelands in the sagebrush steppe in the Pacific Northwest area of the United States. Two species of weevils, *Phrydiuchus tau* Warner and *Phrydiuchus. spilmani* Warner, were introduced from Europe as classical biological control agents. Only *P. tau* established and was widely redistributed throughout the region. Our observations show that, after the establishment and population increase of the weevil, densities of Mediterranean sage decreased at three of the four initial release sites and subsequently at 17 of 25 weevil release sites where plant densities dropped 2–5 orders of magnitude from $\geq 1/m^2$. Level of control appears to be associated with a combination of plant community type, disturbance and grazing intensity. The decline in weed density was most apparent in the sagebrush steppe community with light to no grazing. In comparison, salt desert scrub, annual grass dominated and heavily grazed communities showed little change in Mediterranean sage density over 25 years. This is the first report of successful biological control against Mediterranean sage.

***Keywords***: successful management, range improvement, weevil, *Phrydiuchus tau*.

## Introduction

### Target plant

Mediterranean sage, *Salvia aethiopis* L. (Lamiaceae), is a pungent herbaceous monocarpic, biennial weed, naturalized in the United States from its native range in southern and southeastern Europe (Tutin *et al.*, 1972; Mijatovic, 1973; Davis, 1975; Roche and Wilson, 1999). The plant has been observed and studied in xeric ruderal habitats in Yugoslavia, Bulgaria, Greece, Turkey and Iran, and was occasionally observed in similar habitats in France, Italy and Spain (Andres and Drea, 1963).

Mediterranean sage and several of its allies have been imported and cultivated in the United States for use as ornamentals and medicinal herbs (Bailey, 1935). Mediterranean sage was first reported in North America from California in 1892 (Howell, 1942) and was suspected of being introduced as a contaminant in alfalfa seed (Dennis, 1980). It has since spread and become a serious range pest in Oregon, California and Idaho and a minor problem in Washington, Colorado, Texas and Arizona (Wilson *et al.*, 1994; Coombs and Wilson, 2004). The major infestation in the United States occurs in the Goose Lake Basin in southern Lake County, OR, and northern Modoc County, CA (Andres *et al.*, 1995). Overall infestations were estimated at 510,000 ha in seven Oregon counties (Radtke and Davis, 2000), 2800 ha in northeastern California, 1600 ha in northern Idaho (Wilson *et al.*, 1994) and 120 ha in Colorado (Mowrer, 1996). Infestations have also been recorded in South Dakota, Arizona and Texas.

Mediterranean sage reproduces only by seed, sprouting in the fall or spring after rains provide adequate soil moisture for seedling survival. Young plants overwinter as rosettes and typically bolt in late spring and flower mid-June to early July. Smaller rosettes may overwinter for a second year without flowering (Wilson and McCaffrey, 1993). Mature plants grow to 20–90 cm tall, producing up to 100,000 seeds. Seeds are dispersed during the late summer and fall after the stalks break off and are blown about as tumbleweeds.

In Oregon, the majority of Mediterranean sage occurs in early successional communities in the sagebrush

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steppe, upper salt desert shrub and disturbed sites dominated by annuals (e.g. rangelands and pastures overgrazed by livestock, abandoned farm land, construction sites, rights-of-way and burned areas). This non-toxic unpalatable plant displaces livestock forage and was estimated to cause $1.05 million in annual losses in Oregon (Radtke and Davis, 2000).

Mediterranean sage can be a difficult plant to manage. Foliar herbicides are less effective due to the woolly leaves, which can act as barriers reducing chemical absorption. Furthermore, the plant occurs on low-value land, and in conjunction with high costs and difficulty of control, it was targeted for biological control by the US Department of Agriculture Agricultural Research Service (USDA-ARS) in 1958 (Andres and Drea, 1963). The project was initiated at a time when it was thought that the weed would have a major impact on rangelands throughout the Great Basin in the American northwest.

## Biological control agent

Foreign exploration to search for natural enemies of Mediterranean sage was conducted in Turkey and Iran. A weevil, later described as *Phrydiuchus tau* Warner (Coleoptera: Curculionidae), was found (Andres and Drea, 1963; Andres and Riza, 1965; Andres, 1966; Warner, 1969). A second species, *P. spilmani* Warner (Warner, 1969), was found feeding on *Salvia verbenacea* L. in Italy. During feeding trials, *P. spilmani* also accepted *S. aethiopis* as a host.

Both species of weevils were introduced in the United States as classical biological control agents for Mediterranean sage (Andres *et al.*, 1995). A total of 631 adult *P. spilmani* were collected in Italy and released in Oregon from 1969 to 1970. Although several recoveries of adults were made, the weevil failed to establish (Andres *et al.*, 1995). A total of 1650 adult *P. tau* were introduced from Yugoslavia and released in Oregon from 1971 to 1973 (Andres *et al.,* 1995). *P. tau* readily established, and the sites provided surplus weevils that were shipped to California in 1972 (Andres *et al.*, 1995), Idaho in 1979 (Wilson and McCaffrey, 1993) and Colorado in 1992 (Mowrer, 1996). A limited local redistribution began in Oregon and California in 1976 (Andres *et al.*, 1995), followed by an intensive redistribution program in Oregon by the Oregon Department of Agriculture (ODA) from 1979–1983 (Coombs *et al.*, 1996; Turner and Coombs, 1996).

The life history of *P. tau* was studied as part of the process of assessing its efficacy as a biological control agent. The weevil is univoltine, producing one generation per year. In the fall, females oviposit along the midribs and petioles on the undersides of rosette leaves (Andres, 1966; Wilson and McCaffrey, 1993; Coombs and Wilson, 2004). The newly hatched larvae penetrate the plant epidermis and tunnel down the leaf petiole into the root crown. Larval feeding can sever vascular tissues of the root crown, which results in the suppression of flowering and seed production. Severe larval feeding can result in plant death (Andres, 1966). In the spring (June), the mature larvae exit the rosette and construct a spherical chamber of soil particles in which they pupate. Adults emerge after several weeks and feed on the foliage and flowers before entering a period of summer dormancy (aestivation) until fall rains stimulate plant growth. In the fall, adults feed, mate and deposit eggs on rosettes (Andres, 1966; Wilson and McCaffrey, 1993).

Biological control has come under some scrutiny because of the lack of monitoring efficacy and non-target effects (McEvoy and Coombs, 2000). To document the overall efficacy of the Mediterranean sage biological control project, we determined that there was a need to conduct additional evaluation beyond the original release site studies. Our project objectives were to: (1) continue monitoring the original four ARS release sites, (2) assess efficacy at early ODA regional release sites, (3) document trends of Mediterranean sage density, (4) document the relationship of *P. tau* numbers to plant density and (5) compare trends of plant density to site characteristics and land use.

## Methods and materials

### Original release sites

Initial observations of plant and insect interactions were conducted by Andres and cooperators in southern Lake County, OR, near the town of Lakeview. The climate is characterized by wet winters and dry summers. The average annual precipitation in the Lakeview area is 36 cm. Four sites served as the original release sites for *P. tau*. The criteria for selection were heavy infestations of Mediterranean sage (>1 $m^2$), and representative of the variety of plant communities and land use in the area. Three sites: (1) Geyser, about 1.5 km north of Lakeview; (2) Cottonwood, 13 km west of Lakeview and (3) Killer Hill, 6.5 km south of Lakeview, were classified as sagebrush steppe, encompassing dense to open grassland intermixed with dense to open shrub. The fourth site, Dick's Creek, 24 km north of Lakeview, is in a yellow pine-shrub forest that was logged 10 years before the release of *P. tau*. Unfortunately, several years of data from the ARS studies were lost during a relocation of the USDA-ARS offices at the Albany, CA facility. Furthermore, the Killer Hill site was infrequently visited because it was difficult to relocate.

Periodic visits were made to the four original release sites, usually in early November to (1) estimate the density of rosettes per square metre, (2) conduct a timed count of the number of adult weevils observed per observer hour and (3) estimate percent ground cover by rosettes. No data were taken between 1980 and 1991. Plant density and percent cover were determined by using a 1/4-$m^2$ quadrat frame placed on the ground every 4 m along established transects. Twelve readings

were made along each cardinal direction for a total of 48 plots per site per visit. Weevil counts were obtained by counting the number of adults observed per minute in each transect to determine number of weevils per observer hour.

## Regional release sites

After the establishment of *P. tau* at the original release sites, a programme of collection, redistribution and monitoring was conducted by ODA. By November of 1976, populations of *P. tau* at the Cottonwood site reached >100 adults collected per hour. Weevils from that site were redistributed throughout infestations in Oregon and California. In the following years (1979 to 1983), ODA and cooperators made releases of *P. tau* throughout the region. The objective was to release weevils at all heavily infested townships and in each major drainage system. Some collection and redistribution efforts were made by ODA during later years at new and isolated infestations and for releases in other states (Coombs and Wilson, 2004).

The collections of *P. tau* adults for redistribution were primarily conducted during the mating period in November. Weevils were sorted into groups of 100 adults and placed into containers provisioned with tissue paper and host plant foliage. Releases occurred within several days of collection at Mediterranean sage infestations ≥0.5 ha and a minimum density of 1.0 $m^2$.

In the summer of 1995, a field survey was conducted at 25 sites where *P. tau* had been previously released by ODA from 1979 to 1983. Only sites that could be positively located from maps on the original release forms were selected. Each site was inventoried for (1) estimating plant density by using 1 $m^2$ quadrats or actual counts at low densities within 1000 $m^2$, (2) determining the number of adult *P. tau* observed per minute, (3) assessing present land use and degree of grazing, rated as none, light (<50% use) and heavy (>50% use) and (4) documenting plant community type. The apparent degree of control of Mediterranean sage based on density of plants square metre was categorized as: poor, ≥1.0; fair, 0.99–0.1; good, 0.09–0.01; and excellent, ≤0.009. The sites were periodically sampled from 1999 to 2006 to determine the stability of control and to assess variability within plant populations.

# Results

The weevil, *P. tau*, established at all four of the original ARS release sites. Weevils from these sites were later collected and released at numerous infestations in the region. Since the inception of the project, density and percent cover of Mediterranean sage has declined by several orders of magnitude at most sites in the Goose Lake Basin but not in the lower and drier Abert Lake Basin. The following are some of the early results that depict the interaction of weevil-plant dynamics through time at the original release sites and regional release sites. Plant community types and land use interactions will be analyzed in future works.

## Original release sites

Original plant density at the release sites varied from 3.2 to 12.4 $m^2$ at the time of weevil release (Fig. 1).

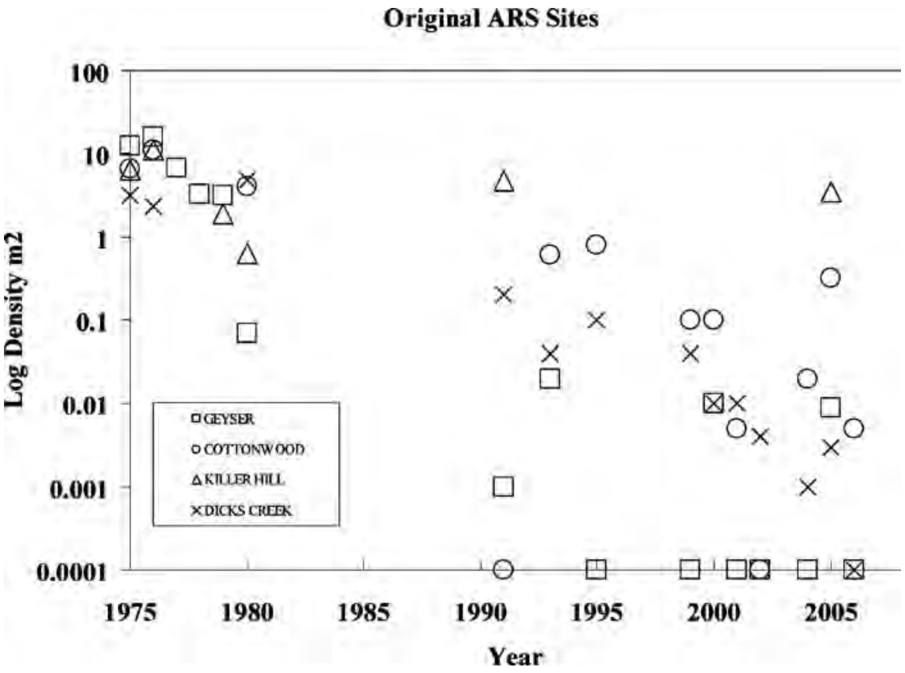


**Figure 1.** Density changes $\log_{10}$ of Mediterranean sage at the four original USDA-ARS study sites. Triangles: Killer Hill site, a heavily over-grazed site that has changed little since the release of *Phrydiuchus tau*. Zero data were entered as 0.0001.

Subsequent monitoring of the Mediterranean sage density showed a slight downward trend during the first 5 years among the four sites (Fig. 1). By 1992, plant densities had decreased to less than 1 $m^2$. From year 2000 on, plant densities remained less than 0.01 $m^2$ at all sites, except Killer Hill, which was heavily grazed each winter by livestock.

We measured plant density, cover and weevil numbers at the Geyser site and found cover and weevils correlated with plant density (Fig. 2). The weevil population increased to more than 100 per observer hour at two of the sites from 1976 to 1978. After this peak, weevil numbers dropped to 1.3 per observer hour by 1993. By 1993, plant density had dropped to 0.02 $m^2$. No plants or weevils were found at the Geyser site during the July 1995 visit (Fig. 2). In 1999 and in 2000, several plants with adult feeding were observed but none in 2001 samples, as the site had been recently mowed and sprayed with herbicides to control other species of weeds. The Geyser site was representative of the trends that we observed in Dick's Creek and Cottonwood sites, in that weevil numbers increased and later decreased as plant density decreased. However, the heavily grazed Killer Hill site changed little in 25 years (Fig. 1).

## Regional survey

More than 120 releases of *P. tau* occurred from 1979 to 1983, which were collected at the original ARS release sites. After these releases, casual inspections by various cooperators revealed that the weevil was widespread throughout the Mediterranean sage infested area. In 1995, we determined that 25 release sites were amenable to quantitative monitoring to document changes over time (Table 1). Variations in Mediterranean sage densities appear to be associated with plant community type and community disturbance history (grazing intensity, fire, road construction and agriculture). The lowest Mediterranean sage densities observed were at sites that consisted of perennial grass/shrubs with no-to-light grazing. Conversely, we observed higher densities of Mediterranean sage at sites characterized by salt desert scrub and annual grasses. At sites with heavier grazing pressure ($\geq$50% biomass removal of perennial grasses), control of Mediterranean sage (0.5–0.05 mature plants/$m^2$) was better in communities with a strong perennial grass component. The poor control of Mediterranean sage in the ungrazed areas was due partly to historic heavy grazing, having reduced the perennial grasses, and more to recent fires, which allowed the invasion of annual grasses. Again, the lack of competition and resetting the successional clock were likely factors.

In the 1995 survey, 12 sites were not grazed, five were lightly grazed and nine were heavily grazed (Table 1). It appears that heavy grazing reduces the amount of plant competition against Mediterranean sage plants weakened by the biological control agent. Caution should be used when comparing these sites, as our inventory was not designed to cover an equal number of sites in each category.

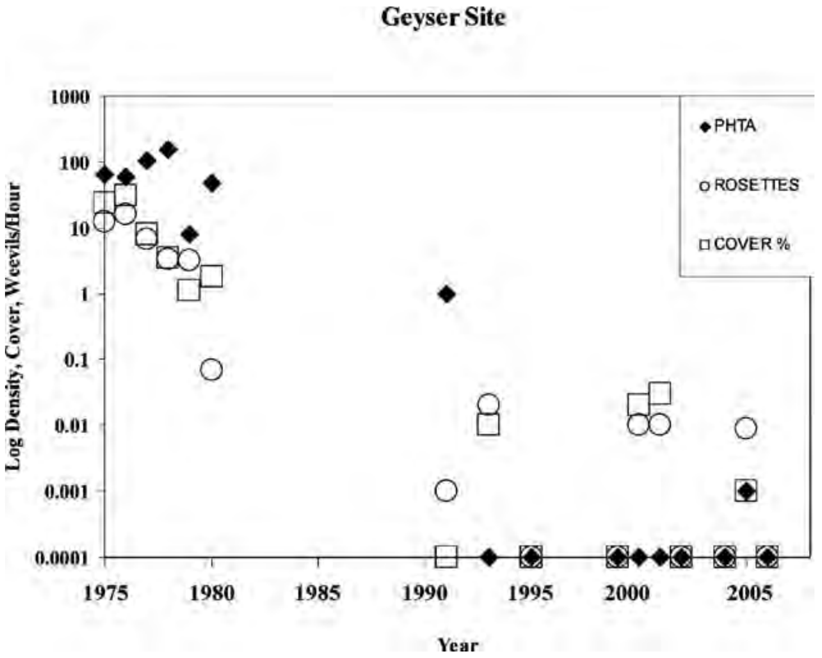


**Figure 2.** Changes in $\log_{10}$ of plant density, percent plant cover and numbers of *Phrydiuchus tau* (PHTA) per observer hour over time at the original USDA-ARS Geyser site. Zero data were entered as 0.0001.

**Table 1.** Comparison of the level of biological control of Mediterranean sage, plant community type and grazing levels at 25 *Phrydiuchus tau* regional release sites in Oregon in 1995.

| Control level[a] | Number of sites | Community type[b] | Grazing intensity[c] | | |
|---|---|---|---|---|---|
| | | | Heavy | Light | None |
| Excellent | 6 | Shrub/perennial grass | 0 | 4 | 2 |
| Good | 4 | Shrub/perennial grass | 1 | 0 | 3 |
| Fair | 4 | Shrub/annual | 3 | 0 | 1 |
| | 3 | Salt scrub/annual grass | 2 | 1 | 1 |
| Poor | 2 | Shrub/perennial grass | 1 | 0 | 1 |
| | 4 | Shrub/annual | 2 | 0 | 2 |
| | 2 | Salt scrub/annual grass | 0 | 0 | 2 |
| Total | 25 | | 9 | 5 | 12 |

[a] Control level = average mature plants per square metre; poor, ≥1; fair, 0.99–0.1; good, 0.009–0.01; excellent, ≤0.009.
[b] Community type – based on dominant species: shrub = *Artemisia tridentata, Purshia tridentata*; salt scrub = *Sarcobatus vermiculatus, Atriplex confertifolia*; perennial grass = *Agropyron cristatum, Festuca idahoensis*; annual grass = *Bromus tectorum, Taeniatherum caput-medusae.*
[c] Grazing intensity – utilization of above ground biomass: heavy, ≥50%; light, ≤50%.

Of the regional release sites, 12 were dominated by perennial grasses and shrubs and 13 had a large component of annual grasses (Table 1). Four of the sites had no detectable level of Mediterranean sage (three were shrub/perennial grass communities and one was in agricultural production). In contrast, the highest density of Mediterranean sage (≥1.0/m$^2$) remained in salt desert scrub and shrub/annual grass communities, irrespective of grazing. When compared to the original minimum density estimate 1.0 mature plant per square metre, 68% of the survey sites showed reductions of Mediterranean sage of one to three orders of magnitude. We are more concerned about the numbers of mature plants at sites because they produce seed and contribute to rising generations.

Among the 25 sites visited in 1995, 16 sites were periodically re-inventoried through 2006. We observed two distinct patterns in Mediterranean sage density through time based on plant community type (Fig. 3). One pattern was a precipitous drop in Mediterranean sage from the initial estimated plant density (≥1 m$^2$; Fig. 3). The second pattern exhibited a stable trend of Mediterranean sage density over time in plant communities that had high components of annual grasses, mostly due to recent disturbances (fire and heavy grazing). From 1995 through 2006, Mediterranean sage con-

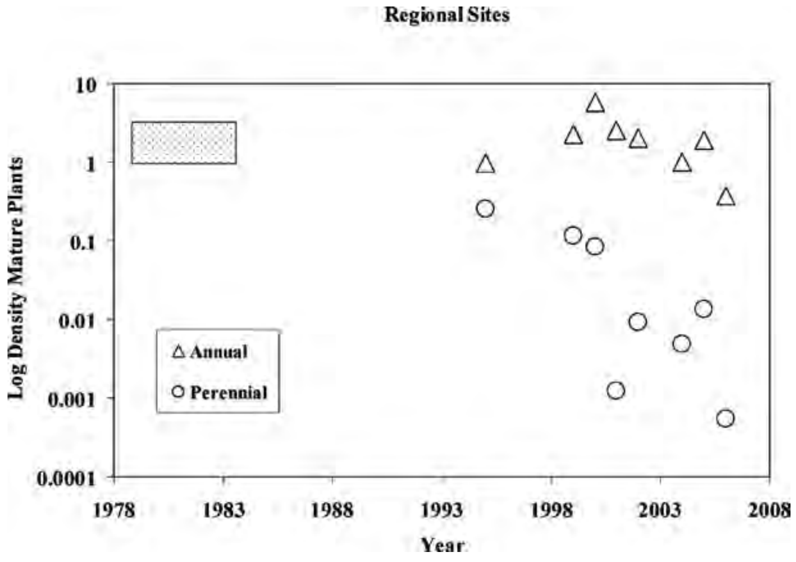


**Figure 3.** Changes in plant density (log$_{10}$) of ODA regional release sites ($n$ = 25) over time. The *large rectangle* represents a conservative estimate of density of Mediterranean sage at release of *Phrydiuchus tau* and during the 5 or 6 years after release of *P. tau*. Circles represent salt desert scrub sites in the Abert Lake Basin with high annual grass component and triangles represent Goose Lake Basin sites with high perennial shrub/grass components. Zero data were entered as 0.0001.

tinued to decline to the point that, by 2006, nine sites exhibited excellent control (≤0.009 mature plants/m$^2$). Most severe infestations typically have two to five mature plants and five to 15 rosettes per square metre. As original mature plant density estimates were ≥1.0/m$^2$, our conservative appraisal of control may underestimate the effectiveness of *P. tau*. By 2006, 17 of the 25 ODA regional release sites inventoried showed a reduction in Mediterranean sage density of more than 90%, with nine sites near 99%. One mature plant per square metre was considered as the economic threshold level to justify control measures.

In 2005, after a period of heavy late spring rains, Mediterranean sage populations in the annual grass dominated salt desert scrub community in the Abert Lake basin flourished (1.5/m$^2$), and we expected to see a large increase of Mediterranean sage the following year. However, outbreak-level populations of *P. tau* severely defoliated rosettes and flowering plants (50–90% defoliation). Monitoring in 2006 showed an average reduction of mature plants to 0.1 m$^2$, which we attributed to the heavy impact by the weevil.

## Discussion

Smith and DeBach (1942) identified three criteria essential for evidence of successful biological control. The Mediterranean sage biological control project meets two of those criterions. First, the densities of Mediterranean sage declined after the introduction, establishment and increase of *P. tau*. In fact, two sites used by ODA personnel to collect *P. tau* in the late 1980s were no longer viable in 1994 because the host plants were nearly eliminated. Second, the levels of Mediterranean sage for the most part have remained at low levels after the establishment and increase of *P. tau*. The third criterion, to show that the target weed would return to its original density when the weevils are removed, remains to be experimentally tested.

Populations of *P. tau* established and increased at the four original study sites, later followed by a decrease in the density of Mediterranean sage and eventually a decrease on *P. tau*. Following a lag time of 10 to 20 years after redistribution of the weevil, reductions of Mediterranean sage density occurred throughout much of the Lakeview area. The majority of landowners, managers and county extension agents we consulted agreed that the overall density of Mediterranean sage in the Lakeview Valley was much lower than before *P. tau* was released.

Our studies suggest that larval destruction and weakening of Mediterranean sage rosettes, supplemented with the stress of competing perennial vegetation, were the main causes of control. Plant competition appears to play an important role in enhancing Mediterranean sage control. Best control occurred in those communities with a strong perennial grass component. Conversely, despite weevil presence, wherever recent fires and heavy grazing had reduced the vigour of competing grasses, the level of Mediterranean sage remained troublesome or decreased slightly. Mediterranean sage sites in some salt desert shrub and annual grass communities (dominated by *Bromus tectorum* L. and *Taeniatherum caput-medusae* L.) showed little change from their original densities. Plant competition and other environmental stress factors, including limited rainfall that can impact Mediterranean sage (Wilson *et al.*, 1994), contributed little to control before introduction of *P. tau*. Some fluctuation in the density of Mediterranean sage is to be expected, but most sites under good to excellent control have not returned to initial pre-*P. tau* levels. We estimate that 68% of the original release sites were controlled, averting an annual forage loss of about $0.8 M over 508,000 ha.

Despite successful biological control at many sites in south central Oregon, Mediterranean sage continues to flourish and infest new and isolated areas in northern Oregon and several western states. Although many of those smaller infestations have been targeted for intensive control, *P. tau* has been released and recovered at some. Impacts of *P. tau* in those areas should be monitored. The ability for Mediterranean sage to spread and prosper at disturbed sites suggests that additional natural enemies should be sought to improve control. Mediterranean sage is seed limited; therefore, seed destroying biological control agents may prove more effective in areas where *P. tau* is insufficient.

Without long-term monitoring, the biological control of Mediterranean sage may have been forgotten and its efficacy never demonstrated. This can be a difficult task when old biological control projects span across the careers of multiple scientists, agency priorities and funding commitments.

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# Preliminary results of a survey on the role of arthropod rearing in classical weed biological control

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*Summary*

The rearing of arthropods is an essential but sometimes neglected and underestimated part of a classical weed biological control programme. Success in rearing is usually a pre-requisite to conducting host-specificity tests, obtaining enough individuals for initial field release or, later, for large-scale implementation. Although most biological control researchers can list situations where agent development has been stopped or slowed due to rearing difficulties, failures seldom get reported in the literature, thus preventing us from gauging the extent and relevance of rearing issues. To rectify this, a questionnaire was developed to investigate the prevalence of rearing problems in weed biological control programmes and to classify their occurrence according to a list of variables (e.g. taxonomy, biological features, genetic issues and researcher/programme attributes). The questionnaire was sent to 80 researchers from eight countries; 65% responded, generating 79 useful responses. Results confirm that, of the challenges faced in programmes, rearing is the most prevalent (56% out of ten possible general problem categories). The most common rearing problems encountered were conditions that were not conducive to mating and/or oviposition (30% of reported arthropod cases) or development (22% of reported arthropod cases). Our results identify key areas for rearing improvement, thus contributing to increased weed biological control project successes.

***Keywords***: international survey, project challenges, rearing difficulties.

## Introduction

The ability to rear arthropods for classical weed biological control programmes is a topic seldom formally addressed within our scientific discipline, yet it touches all stages of a programme and has the potential to seriously affect its progress and direction. Rearing can ensure a reliable source of arthropods for either host-specificity testing or initial field releases when agents are rare in their place of origin (Blossey *et al.*, 2000). Furthermore, because host-range testing relies on arthropod behaviours that also are required for rearing (e.g. mating, oviposition), the testing of these arthropods can be greatly hindered or prevented if they cannot be reared in a laboratory environment (Palmer, 1993; Marohasy, 1994; Klein, 1999). Sole reliance on field-collected arthropods from the country of origin for either host-specificity testing or field release is risky. For instance, events that eliminate a field population of arthropods used in testing could set back a programme for years, at great cost. This could be averted if a reared colony of the organism is available. There also is less control over factors that may influence host choice (e.g. prior host experience, readiness for host acceptance; Marohasy, 1998) when field-collected arthropods are used directly in host-specificity tests.

Once an arthropod has been given regulatory approval for release, the possession of a reared colony can greatly aid in achieving successful establishment. This is especially true for arthropods that may be susceptible

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to Allee effects (e.g. ability to find mates), demographic stochasticity or catastrophic environmental events if released in small numbers (Hopper and Roush, 1993; Grevstad, 1999). It also is preferable for the sake of risk management to release agents from a reared colony of known taxonomic identity, purity and genetic source to reduce the possibility of unwanted inclusions such as other species or parasitized or diseased individuals. Although exemptions are possible, a regulatory condition of the Australian permitting system is that the agent must undergo a minimum of one generation in containment before release to avoid such hazards. Other countries, or laboratories within countries, may also attempt to follow this practice for precautionary reasons. Thus, the inability to rear an agent in the laboratory environment may present a direct impediment to its release.

The ability to rear weed biological control agents also influences the widespread implementation of successful agents. Typically, arthropods that are easy to rear and demonstrate rapid population increases under artificial conditions are the most amenable to mass production (Grossrieder and Keary, 2004). Compared to the mass production of entomophagous biological control agents for augmentative use (Thompson, 1999), the development of quality-controlled, mass rearing techniques for classical weed biological control arthropods is still in its infancy. However, recently, there has been a growing effort to experimentally develop methods for laboratory mass rearing of weed biological control agents on plants (Visalakshy and Krishnan, 2001) or artificial diet (Blossey *et al.* 2000; Wheeler and Zahniser, 2001; Goodman *et al.*, 2006; Raina *et al.*, 2006) or within scaled-up outdoor nurseries (Story *et al.*, 1994, 1996; Julien *et al.*, 1999; De Clerck-Floate *et al.*, 2007).

Although examples of failures or difficulties in arthropod rearing are well-recognized, these are seldom reported in the literature (however, see Palmer, 1993; Klein, 1999). The arthropods involved are either dropped from or reduced in priority on candidate lists, even if they show potential as effective agents. To determine the prevalence and types of rearing problems within the field of classical weed biological control, an international survey of researchers was conducted. This paper summarizes key, preliminary results and provides some perspective on how rearing can be managed to improve the science and implementation of weed biological control.

## Materials and methods

A questionnaire on the role of arthropod rearing in classical weed biological control programmes was prepared through collaboration and piloted among six colleagues to ensure that the questions were clearly stated for the information requested. The questionnaire was divided into subsections: (1) general background on the researcher – name, location, years and type of experience in classical weed biological control; (2) programme-specific information (i.e. per weed) – duration of programme, number of arthropod species available on the weed in its place of origin, number of arthropod species tested, released and established, indication of avoidance of arthropod groups because of rearing issues, role of release size on arthropod agent establishment, role, prevalence and type of mass rearing in programmes, prevalence of monitoring for arthropod quality in reared colonies, evidence of genetic management during rearing (i.e. measures taken to reduce likelihood of inbreeding), a detailed list of all arthropod species either used or considered for biological control of the weed, with information on taxonomy, feeding guild and any problems encountered during development or consideration of the agent. The arthropod species-specific information became a large and key component of the data set and allowed analyses by family, order, feeding guild, 'general problems' encountered in the development of each agent (e.g. 'ability to rear'; Table 1), specific 'rearing problems', if they occurred (Table 2) and whether a species was rejected or given lower

**Table 1.** General problems encountered in classical weed biological control programmes and their frequency of occurrence.

| General problems for weed biocontrol programmes | Count | $n$[a] | Frequency (%) |
|---|---|---|---|
| Ability to rear | 246 | 439 | 56 |
| Ability to establish agent in the field | 72 | 362 | 20 |
| Collecting sufficient specimens during exploration stage | 85 | 444 | 19 |
| Ability to elicit appropriate oviposition or feeding behaviours during host-specificity testing | 50 | 411 | 12 |
| Detecting/confirming establishment at release sites | 35 | 322 | 11 |
| Inadequate taxonomy | 40 | 444 | 9 |
| Ability to accurately document agent impact on the target weed | 28 | 319 | 9 |
| Obtaining/collecting sufficient agents for general distribution | 27 | 321 | 8 |
| Other | 24 | 315 | 8 |
| None | 62 | 319 | 19 |

[a] '*n*' for each problem varied depending on whether the programme stage allowed for encounter of the problem, and thus the inclusion of appropriate cases in the calculation of frequency.

**Table 2.** Specific problems encountered when rearing arthropods for classical weed biological control and their frequency of occurrence (*n* = 246). Note that more than one problem could arise per arthropod case that was listed.

| Rearing problems | Count | Frequency (%) |
|---|---|---|
| Experimental conditions not conducive to mating and/or oviposition | 74 | 30 |
| Experimental conditions not conducive to normal development | 53 | 22 |
| Long life cycle | 52 | 21 |
| Obtaining appropriate phenological stage of host plant for rearing | 51 | 21 |
| Unknown biology or life cycle | 49 | 20 |
| Field collection of enough individuals to start a laboratory colony | 44 | 18 |
| Host plant nutrition/quality | 34 | 14 |
| Incompatibility with host plant | 33 | 13 |
| Mortality during storage of diapausing arthropods | 28 | 11 |
| Synchronization between northern and southern hemispheres | 27 | 11 |
| Presence of parasitoids | 26 | 11 |
| Presence of pathogens | 20 | 8 |
| Failure to break diapause | 19 | 8 |
| Difficulties with artificial rearing medium | 7 | 3 |
| Inadequate labour or facilities | 6 | 2 |
| Presence of predators | 4 | 2 |
| Inbreeding or genetic adaptation to laboratory conditions reducing quality of colony | 4 | 2 |
| Presence and possible effects of endosymbionts | 4 | 2 |
| Mutualist needed | 3 | 1 |
| Difficulties in distinguishing sexes | 3 | 1 |
| Other | 4 | 2 |

**Table 3.** The stages in classical weed biological control programmes at which rearing problems occurred and the number and frequency of occurrence for each stage (*n* = 85).

| Project stage | Count | Frequency (%) |
|---|---|---|
| (A) Exploration | 16 | 19 |
| (B) Host-specificity testing | 43 | 51 |
| (C) Early release and monitoring for establishment | 20 | 23 |
| (D) Mass production | 4 | 5 |
| (E) General distribution of agents | 2 | 2 |
| (F) Monitoring for impact by agents | 0 | 0 |

priority at any point because of rearing problems. Only summary results from the survey are presented in this paper due to the large volume of information that was obtained. A journal publication presenting the broader results is in preparation.

A total of 80 researchers from eight countries were surveyed. Respondents were asked to complete a separate questionnaire for each weed species with which they had been involved and to only answer questions pertinent to their experience within programmes, as identified by stage (Table 3). Programme stage was taken into account during determination of the prevalence of various general problems or rearing problems by including these data for summary or analysis only if the researcher could have conceivably encountered the problem(s) at their programme stage(s). This controlled for any bias due to stage of programme during data summary. For example, General Problem, ‘Ability to accurately document agent impact’ (Table 1) would only be encountered at stage F of a programme (Table 3); thus, only agents in programmes that had reached stage F were included in calculations involving this problem.

# Results and discussion

## General results

A total of 52 researchers (65%) responded to the survey, generating 84 questionnaires, which represent the majority of active weed biological control programmes worldwide. The countries and number of researchers based in each were Australia (16), South Africa (9), USA (9), Switzerland (6), New Zealand (5), Canada (4), France (2) and Argentina (1). The average experience in weed biological control of the researchers was about 19 years, and the cumulative experience by the respondents was 987 years. Most of the researchers

were based in the introduced range of the plant (54%), some in the native range (21%), while others worked in both (25%).

Of the 79 questionnaires that were included in the analyses (five were not completed properly and were excluded), the median duration of the weed programmes was 17 years. All programmes had completed stage A, exploration and were at least in stage B, host-specificity testing (see Table 3 for a list of stages). The majority of programmes in the introduced range had reached the final stage F (monitoring for impact). In 65% of cases, respondents reported work on a unique target weed, while in 35% of cases, the same species was reported from another country (i.e. work within native vs introduced ranges or two or more countries working on the same weed) or at a different programme stage. Specifically, the same weed was reported twice in eight cases and three times in four cases.

The analysed responses covered a total of 64 weed species located in various climatic zones including; temperate (32 species), temperate/subtropical (5), subtropical (11), subtropical/tropical (9), tropical (6) or temperate/subtropical/tropical (one aquatic species). The majority of target weeds were perennials (52 species) with a few biennials (7) or annuals (5). The number of herbivorous arthropod species occurring on a weed in its area of origin was estimated through the literature, field surveys or both by respondents for 45 of the target weeds. The average number of herbivores was 120 but generally increased from temperate to tropical zones (Fig. 1), which is an expected trend based on studies of arthropod diversity along latitudinal gradients (Price, 1997).

Information on a total of 384 arthropod species (nine eriophyoid mites and 375 insect species) were recorded in the questionnaires. The insects were from 6 orders and 66 families. A few of the arthropods were used for more than one weed target, resulting in a total of 444 cases for data analysis.

## Incidence of rearing problems

Respondents reported many general problems in the development of arthropod agents for weed biological control; by far, the most common was 'ability to rear' with 56% of the arthropod cases listed in questionnaires (Table 1). Other common problems, such as 'collecting sufficient specimens during exploration stage' and 'ability to establish agent in the field', may also be related to the ability to rear, as it is

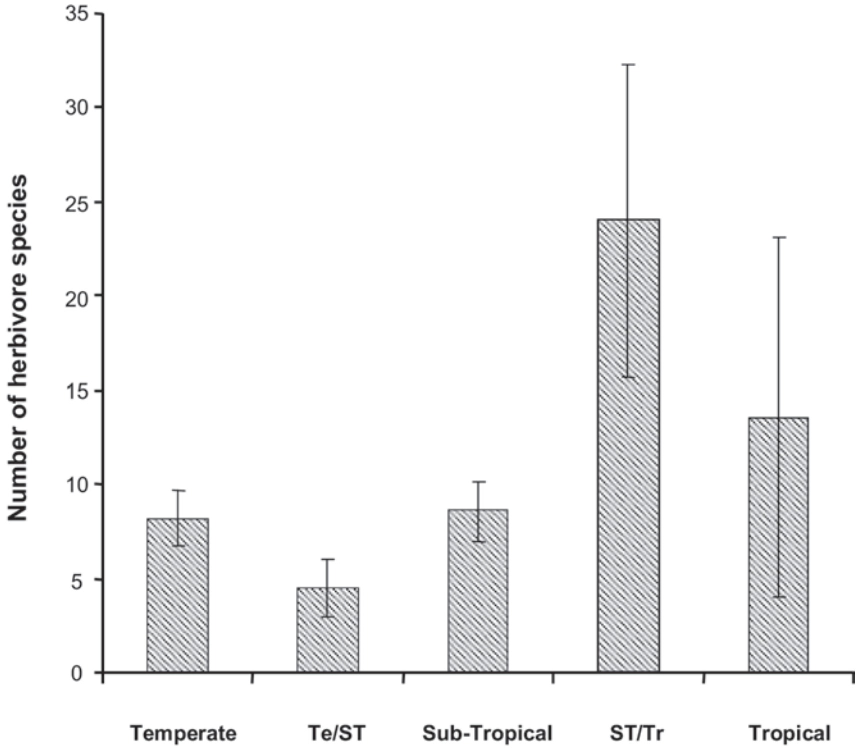


**Figure 1.** Mean number (±SE) of herbivore arthropod species estimated (from field surveys, the literature or both) to occur on a target weed species in its area of origin according to climatic zone. *Te/ST* Temperate/subtropical, *ST/Tr* subtropical/tropical.

suspected that they may not have attained this level of relevance if rearing were easy. For instance, there is evidence that releases at more sites and/or with larger numbers of individuals per release in a geographic area improves establishment success (Hopper and Roush, 1993; Memmott *et al.*, 1998; Grevstad, 1999). It has also been acknowledged that difficulties in rearing may jeopardize agent establishment where there are known hazards to small, founding, field populations (Spafford Jacob *et al.*, 2007). In our questionnaire, 73% (174/237) of answered cases indicated that there were examples within programmes where it was suspected that failure to establish may have been due to small release size.

Specific rearing problems experienced by researchers can be grouped into several categories. The most common were conditions that were not conducive to mating and/or oviposition or normal development of the immature stages (Table 2). Often, appropriate artificial light and temperature conditions must be experimentally identified to induce arthropod behaviours required for successful rearing (Baars, 2001). In addition, very common were problems due to long or unknown life cycles or unknown biology. Issues related to the host plant such as nutrition, quality, incompatibility or providing the correct phenological stage were very common. Obtaining enough individuals to start a colony was a common problem as were problems with synchronization and diapause. Issues associated with organisms such as parasitoids, pathogens, predators, endosymbionts and mutualists were reported less frequently. In some situations, arthropods reared under laboratory conditions simply did not thrive for obscure reasons, which require further investigation (Hoffmann, 1988). For instance, among respondents to this survey, only 2% of cases had 'inbreeding or genetic adaptation to laboratory conditions reducing quality of colony' listed as a rearing problem (Table 2); yet, when they were specifically asked whether they monitor for arthropod quality (e.g., fecundity, fertility) during rearing, only 39% (170/430) of cases were affirmative, and in 44% (190/430) of cases, agents were not monitored at all. This lack of quality control clearly indicates a reduced possibility of identifying genetic problems in rearing, even though they are known to occur (Torres *et al.*, 1991; Wardill *et al.*, 2004). Changes in fecundity or fertility also may indicate the effect of endosymbionts such as *Wolbachia* spp., which are seldom considered in biocontrol programmes (Floate *et al.*, 2006).

The stage in a project in which an arthropod was rejected or given lower priority because of rearing problems was recorded for only 85 cases out of 444 (Table 3). Despite the poor response to this question, the results still show that when decisions are clearly made due to rearing difficulties, most rejections or priority adjustments occur during the host-specificity testing stage. Of note is that 20 species were rejected at the release stage and four at the mass-production stage. These 24 cases may represent very costly situations, as large programme expenses were likely already incurred at the time of rejection.

Rejection or priority adjustment also occurred during 'exploration' when rearing is required for identification, biology and life-history studies and sometimes preliminary host-range tests (Table 3). After the survey, it occurred to us that there may have been a blurred boundary between exploration and host testing, thus inflating the relevance of the host-specificity testing stage as a time when rearing issues surfaced. For instance, if exploration were conducted through quick, infrequent visits to the place of weed origin by remotely situated researchers, rather than from a more permanent base located in the place of origin, then all work conducted in the recipient country may have been grouped under 'host testing'. This ability to clearly distinguish the boundary between these two stages may be a limitation of the survey.

Importantly, identification of rearing difficulties in the early stages of a programme allows for either the deployment of resources to develop needed rearing techniques or the rapid adjustment of programme goals, so that resources can instead be allocated to candidates that are easier to rear (Hoffmann, 1988). Although details on the taxonomic affiliations of difficult-to-rear arthropods will be forthcoming in a future publication, 27% (104/379) of answered cases in our survey indicated that certain taxonomic groups or feeding guilds were initially either avoided or given lower priority due to perceived potential rearing problems. Interestingly, there was a positive linear relationship between the estimated number of species feeding on a weed in its place of origin and the number of agents tested by researchers ($R^2 = 0.605$, $P = <0.001$, $n = 364$). However, there was no correlation between the estimated number of species and the incidence of rearing difficulties ($R^2 = 0.003$, $P = 0.287$, $n = 381$). Thus, there was no indication that, if researchers have more insects to choose from as potential agents, they tend to test those that are easier to rear, or it could quite simply mean that we are poor at predicting at the start of a programme which arthropods will be easiest to rear.

## Conclusions

The preliminary results of our survey indicate that arthropod rearing issues are perceived to be important among classical weed biological control researchers. They have a very important impact in the development of successful agents and therefore on the outcome of programmes. Despite this, there is ample opportunity to improve rearing efforts and methodology.

Where possible, we recommend that rearing be incorporated into the exploration stage of programmes, as suggested by Hoffmann (1988), or as very early studies in the recipient country in cases where exploration is practiced as quick visits for arthropod collec-

tion by remote researchers. Should rearing difficulties occur, emphasis could then be placed on developing laboratory-based rearing techniques. Only then should otherwise promising species with apparently insoluble rearing problems be given lower priority or abandoned as candidate agents before major costs are incurred. The fact that 27% of respondents avoid taxonomic groups that have perceived potential rearing problems may be good for immediate resource management. However, unless species in the 'troublesome' groups are studied, this avoidance will not lead to improved rearing capability, and potentially useful agents may be rejected prematurely.

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# Beginning success of biological control of saltcedars (*Tamarix* spp.) in the southwestern USA

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*Summary*

Saltcedars, *Tamarix* spp., exotic, invading deciduous shrubs or small trees from Asia and the Mediterranean area, have become the most damaging weeds of riparian areas in the western USA. We and our cooperators have obtained highly successful initial control of saltcedar by introducing the north Asian leaf beetle (*Diorhabda* sp., China/Kazakhstan ecotype) at five sites north of the 38th parallel, but they failed to establish farther south. In 2001, we discovered southern-adapted *Diorhabda* beetles on saltcedar and began testing them. In 2004, we released 2408 Greek beetles at Big Spring, TX; by September 2004, they had defoliated two trees, by 2005, 210 trees (0.8 ha) and by 2006, 7.3 ha of the saltcedar stand and 1.4 ha at Cache Creek, California, and had begun defoliating saltcedar at Pecos and Imperial, TX. The Uzbek beetles are increasing rapidly at Lake Meredith, TX and Fukang, China beetles at Artesia, NM, but the Greek and Tunisian beetles have not established near Kingsville in south Texas. We have revised the taxonomy of the five *Tamarix*-feeding *Diorhabda* ecotype/sibling species and predicted their climatic affinities in North America, correlated depletion of stored carbohydrates by beetle defoliation with plant death, developed pheromone attractants, remote sensing, improved release methods and a model of beetle dispersal and estimated possible damage to beneficial *T. aphylla* (Linnaeus) Karsten (athel) in the open field.

***Keywords***: saltcedar, athel, *Tamarix*, biological control weeds, *Diorhabda*.

## Introduction

Western North American riparian ecosystems, beginning in the mid-1800s, have been invaded by exotic saltcedars (Tamaricales: Tamaricaceae: *Tamarix* spp.), shrubs or small trees native in Asia and the Mediterranean area (Baum, 1978). Three species have become serious weeds, *Tamarix ramosissima* Ledebour, *Tamarix chinensis* Loureiro and hybrids between them and other species in the western USA and northern Mexico and *Tamarix parviflora* de Candolle in California (Gaskin and Schaal, 2002, 2003). In addition, *Tamarix canariensis* Willdenow and *Tamarix gallica* Linnaeus (identification uncertain) and their hybrids with *T. ramosissima* and/or *T. chinensis* occur along the Gulf of Mexico coast from Louisiana to Port Isabel, TX but appear less invasive. *Tamarix aphylla* (Linnaeus) Karsten (athel) is a large evergreen, cold-intolerant tree that

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is less invasive and is used for shade and windbreaks mostly in desert areas of northern Mexico and the southern third of the adjoining states in the United States; it is not presently a target for biological control. Saltcedars occupy approximately 800,000 ha of prime bottomlands from Montana into northern Mexico where they severely damage native plant and animal communities and rangeland ecosystems, including that of many endangered or threatened species (DeLoach *et al.*, 2000).

Investigations into biological control began in 1986 by the US Department of Agriculture, Agricultural Research Service (USDA-ARS) at Temple, TX (DeLoach), and from 1991 with our overseas cooperators, R. Jashenko and I.D. Mityaev (Kazakhstan), R. Wang, Q.G. Lu, B.P. Li and H.Y. Chen (China), S. Myartseva (Turkmenistan), D. Gerling (Israel), A. Kirk, R. Sobhian and L. Fornasari (ARS, European Biological Control Laboratory (EBCL, France) and J. Kashefi (EBCL, Greece; DeLoach *et al.* 2004; Carruthers *et al.*, 2008). Carruthers joined the project in 1998, followed by the other cooperators, especially after field releases began. This collaborative project includes scientists and their co-workers at each of the release sites and for specific research.

## Taxonomy and biology of *Diorhabda* spp.

The first natural enemy chosen for introduction and testing in 1992 was the leaf beetle, *Diorhabda* sp. (Coleoptera: Chrysomelidae), from Fukang in northwestern China and Chilik in eastern Kazakhstan. In 2002, other more southern-occurring *Diorhabda* ecotypes were collected. All were identified by leading taxonomic experts as *Diorhabda elongata* (Brullé). Male and female genitalia were dissected from these collections and from some 700 museum specimens from over 370 locations in 37 countries of Asia and the Mediterranean area. J. Tracy (personal observation) found that the specimens fell into the five following ecotypes, corresponding to five previously named species or subspecies (later synonymyzed but which Tracy reassigned as five species; with probable areas of best adaptation in North America): (1) Fukang and Turpan, China and Chilik, Kazakhstan ecotype (deserts north of the 33rd northern parallel); (2) Crete and Posidi, Greece ecotype (Mediterranean climate CA, central TX); (3) Sfax, Tunisia ecotype (southern TX, southern coastal CA, Sonoran Desert, northern Mexico); (4) Karshi and Bukhara, Uzbekistan ecotype (plains grasslands of Oklahoma, New Mexico, Trans-Pecos, TX; Mojave Desert) and (5) coastal Iran ecotype (southern TX, Sonoran Desert), not yet imported.

Tracy's species designations also are supported by other evidence. Cross-mating experiments that measured egg fertility [Thompson (with K. Gardner), New Mexico State University, Las Cruces (NMSU)], agreed with most of Tracy's designations except that the beetles from Crete and Tunisia mated freely for several generations with little fertility decline and Karshi beetles hybridized with both Crete and Fukang beetles, albeit with reduced fitness in both crosses. DNA analyses supported species status for all five ecotypes examined (D. Kazmer, unpublished data). Pheromone analyses revealed that an aggregation pheromone produced by the males consisted of two complex alcohols, which varied in ratio between the four ecotypes from 1:1 to 1:30 (Cossé *et al.*, 2005; A. Cossé, unpublished data). Differences in pheromones between species may partly explain why Tracy did not find hybrids among the many specimens examined from the Old World: The species with different pheromones were not attracted to each other and did not mate but may mate and produce hybrids when confined in cages. Both adults and larvae of *Diorhabda* feed and females oviposit on the foliage of *Tamarix*. The adults overwinter and the larvae pupate, under litter on the soil surface. The larvae have three instars and the life cycle requires approximately 34 days during summer. The beetles have two generations a year in areas north of the northern 38th parallel (Lewis *et al.*, 2003b; Bean *et al.*, 2007a,b) and three to five further south (Milbrath *et al.*, 2007).

## Release, impact and establishment of the China/Kazakhstan ecotype of *Diorhabda* beetles

The biological control program incurred a 6-year delay from the time we were prepared to release the *Diorhabda* beetles in June 1995 until actual release in May 2001. This was caused by the listing of the southwestern willow flycatcher, *Empidonax trailii* Audubon ssp. *extimus* Phillips (which had begun nesting in saltcedar in recent years, mostly in Arizona), as federally endangered (USDI-FWS, 1995). This required the submission of a biological assessment (DeLoach and Tracy, 1997) and consultation with the US Department of Interior-Fish and Wildlife Service (USDI-FWS). The resulting Letter of Concurrence allowed releases only at the ten specified sites in six states, only farther than 160 km from where the flycatcher nested in saltcedar. The first releases were to be in field cages for 1 year and extensively monitor beetle populations and dispersal, damage to saltcedar and any non-target plants and effects on native vegetation and wildlife communities. Populations of this flycatcher have increased greatly, especially at Elephant Butte Reservoir in central New Mexico and at Roosevelt Lake in central AZ after the mid-late 1990s, as willows increased after water level changes, to become among the largest breeding flycatcher populations known, nesting mostly in native willows (Sferra *et al.*, 1997; Moore and Ahlers, 2005).

Overseas observations and quarantine testing at Temple, TX since 1992 and at Albany, CA since 1998 had demonstrated that these beetles were attracted to, fed and completed their life cycle only on *Tamarix* and

rarely on related species of *Frankenia* (order Tamaricales, family Frankeniacae) on which the beetles probably cannot sustain a population in nature (Lewis *et al.*, 2003a; Dudley and Kazmer, 2005). These results led to agreements to release from FWS and the six state Departments of Agriculture and release permits from APHIS-PPQ.

These beetles were released in cages in 1999 and in the open field in May 2001 at ten sites in six western US states: California, Nevada, Utah, Colorado, Wyoming and Texas (DeLoach *et al.*, 2004). They established easily at five of the six sites north of the northern 38th parallel and, by 2006, had defoliated 30,000 ha of saltcedar at Lovelock, NV, 2000 ha at Schurz, NV Delta, UT, and Lovell, WY, and 150 ha (limited by surrounding herbicide and insecticide applications) at Pueblo, CO. However, these beetles did not establish at any of the four sites farther south in California or Texas or farther north in Montana or Oregon. At Lovelock, NV, 40% of the trees died where defoliation occurred twice annually for 4 years and 65% died where defoliated for 5 years (Carruthers *et al.*, 2008; T. Dudley, personal communication; J. Knight, personal communication), caused by gradual reduction in carbohydrate reserves (Hudgeons *et al.*, 2007). Tests by Lewis *et al.* (2003b) and Bean *et al.* (2007a) revealed that the lack of establishment in the southern areas was caused by a requirement of the beetles for 14.5-h summer-day length to avoid premature diapause. This day length occurs in the northern areas but is not reached south of the 38th parallel.

## Discovery, testing, release and impact of southern-adapted beetles

Carruthers, with J. Kashefi (ARS-EBCL, Greece), discovered *Diorhabda* beetles on *Tamarix* in Crete in 2001. They were also found in Tunisia (A. Kirk, personal communication) and Uzbekistan (R. Sobhian, personal communication) in 2002. Testing by Bean *et al.* (2007b) revealed that all of these beetle populations were adapted to the southern areas of California, New Mexico and Texas where day lengths are shorter. Quarantine tests at Temple (Milbrath and DeLoach, 2006a,b) and at Albany (Herr, unpublished data) revealed that all are host-specific to *Tamarix* as is the Fukang/Chilik ecotype. Approvals were obtained from FWS, Texas, New Mexico and California Departments of Agriculture and APHIS-PPQ, and releases began in 2004.

### Releases, establishment and impact through 2007

The first southern-adapted beetles to establish (from Crete) were released by DeLoach (with J. Tracy and T. Robbins) near Big Spring, TX. In April 2004, they released 37 adults and another 171 in July, that with their offspring, defoliated two small trees near the nursery cage in mid-July. Then, they released another 2200 adults in August that together defoliated a large tree near the cage by October 2004. By October 2005, the beetles had defoliated 210 trees covering 0.8 ha, by July 2006, 2.4 ha, and by October 2006, 10 ha.By October 2007, the beetles had dispersed 8.5 km and defoliated trees for 7 km along nearby Beals Creek. Remote sensing by low-level, aerial color photography by Everitt *et al.* (2007) clearly shows beetle-defoliated areas, permitting estimates of the areas of canopy that have been defoliated. A mathematical model is under development to describe the advancing wave-form of beetle dispersal during each generation (J. Sanabria, personal communication).

The Crete ecotype also was released at 20 other locations in the Colorado River watershed in west Texas during 2005 to 2007 as part of a study by Knutson and Muegge (with E. Bynum) to develop optimum release methods. The beetles appear to be established at some sites. Along the Pecos River, they released approximately 500 adults from a nursery cage near each of Pecos and Imperial, TX, in early August 2006; by fall 2007, the beetles had defoliated approximately 500 trees along 1 km of the river near Pecos and had dispersed along 400 m of the river near Imperial. The Crete beetles failed at Seymour and Kingsville, TX, and increased and then declined at Lake Meredith, TX, and Artesia, NM. Crete beetles released in 2004 at Cache Creek (near Rumsey, CA) by Carruthers and Herr (unpublished data) established on *T. parviflora* in that area, increased rapidly during 2006, and by October 2007 had defoliated nearly all saltcedar for 32 km along the creek.

In the meantime, Michels (with V. Carney and E. Jones) released the *Diorhabda* ecotype from Posidi Beach (near Thessaloniki), Greece at Lake Meredith, TX, where they increased initially but then declined. The Uzbek ecotype, also released at this site, is increasing. Releases of Posidi beetles near Carlsbad, NM by O'Meara, and the Turpan China ecotype in southeastern Colorado by Eberts failed, probably because the sites flooded. The Fukang beetles released at Artesia, NM by Thompson (with K. Gardner) have adapted to shorter day lengths and appear to have established. Tunisian beetles were released in separate areas from the Crete beetles by Moran near Kingsville; both Crete and Tunisia beetles reproduced well in field nursery cages and in sleeve bags but failed to establish in the open field at this site.

### Possible causes of failure to establish

The strong influence of short summer day length on induced premature diapause and failure to overwinter in southern areas was demonstrated by Bean *et al.* (2007a,b) with the Fukang/Chilik ecotype. In addition, distributional patterns of *Diorhabda* appear to be influenced by biome differences (e.g. desert versus maritime and continental versus maritime climates), latitude and elevation (J. Tracy, personal communication). Also important is the selection of sites unlikely to flood when

pupae or overwintering adults are on the soil surface and, of course, that will not receive herbicide or insecticide applications, mechanical controls or be burned or harmed by human disturbance. Attack on the beetles by arthropod predators in the trees or by ground beetles, mice, etc. on the ground may have caused some failures, which might be improved by selecting sites with simple vegetation communities that produce fewer insects that attract predators. At some locations, the rapid rate of increase of the *Diorhabda* beetles such as at Cache Creek, California, has allowed them to overcome predator attack. In addition, numbers released (which may influence production of sufficient concentrations of the beetle aggregation pheromone), cage type, time of year and the match of *Diorhabda* ecotype/species and *Tamarix* species/hybrids present all seem important to successful establishment. Differences in pheromone concentrations could explain differences in reproduction in cages vs field. At some sites, beetles confined in cages mated and larval populations developed well, but after being released into the open field, the adults dispersed widely and the pheromone concentration may have been too low to allow mate finding. If populations are high, the beetles often migrate in swarms, which may maintain the necessary pheromone levels.

## Releases: Rio Grande, western Texas/ Mexico and non-target effects on athel

The largest and most damaging stands of saltcedars are along the Colorado River of Colorado, Utah, Arizona, California, and Mexico and the Rio Grande of New Mexico, Texas, and Mexico. We refrained from releasing *Diorhabda* sp. along the US–Mexican border because of Mexican concerns, including the potential for beetle attack on non-target athel (*T. aphylla*), which the *Diorhabda* beetles had attacked to some extent in our earlier laboratory and field-cage tests (Lewis *et al.*, 2003a; Milbrath and DeLoach, 2006a,b; Herr *et al.*, unpublished data). However, in preparation for possible agreement with Mexico for the release of the beetles along the Rio Grande, DeLoach (unpublished data) conducted overwintering cage tests along the Rio Grande between Presidio and Candelaria in western Texas with the Crete, Tunisia and Uzbek *Diorhabda* ecotypes, beginning in November 2006. All populations overwintered, although survival of the Crete beetles was slightly higher. Unfortunately, we then discovered that the Tunisian and Uzbek ecotypes had hybridized with the Crete ecotype in the outdoor cage cultures maintained at Temple, TX, and we destroyed these beetles before release.

Uncaged, open-field tests were conducted at two locations in Texas to compare *Diorhabda* host selection for and damage to saltcedar, athel and *Frankenia*. At Big Spring in 2005, approximately 1- to 1.5-m-tall potted athel and local saltcedar and 10-cm-tall *Frankenia* plants were transplanted together in ten 1-m-diameter plots into an old saltcedar stand (4–6 m tall) being defoliated by Crete beetles released the year before. At Kingsville, Moran released over 6000 Crete and Tunisia beetles from 2004 to 2006 into an isolated stand of saltcedar previously inter-planted with 25 athel trees (both 3–4 m tall) and also onto large (10–12 m tall) roadside athel trees located 40 to 60 km away with no saltcedar present (no beetles were previously present at either site). The saltcedar at Kingsville is a hybrid between *T. canariensis*, *T. gallica*, *T. ramosissima* or *T. chinensis*, to which the beetles were not strongly attracted in outdoor-cage tests at Temple (Milbrath and DeLoach, 2006 a,b).

At Big Spring, the beetles attacked only the saltcedar transplants from June to late August, but then, when populations increased to high levels, they attacked both saltcedar and athel. In 2005, DeLoach *et al.* (unpublished data) counted a total of 1711 adults on the saltcedar test plants, 588 on athel (34.5% of the total adults counted) and four adults on *Frankenia*. They also counted 170 egg masses on saltcedar, 30 on athel (15.0% of the total) and none on *Frankenia*. At Kingsville, Moran (unpublished data) found that Crete and Tunisia beetles developed and reproduced in sleeve bags on both saltcedar and athel but laid two- to fourfold more eggs on saltcedar. When adults were released from the sleeve bags, they laid two- to tenfold more eggs and produced many more larvae on saltcedar than on athel within the first 2 weeks of release and never established on athel. However, the beetles did not establish on saltcedar at Kingsville either, probably because the *T. canariensis* hybrid dominant there was less attractive to them. When several hundred adults were placed in sleeve bags on the roadside athel plants, they produced larvae that defoliated branches inside sleeve bags, but when released, they simply flew away, apparently in search of a more attractive saltcedar species or hybrid, and never established a population on athel. In similar open-field tests at Cache Creek, California, in 2007 (Herr and Carruthers, unpublished data), the Crete beetles defoliated potted *F. salina* plants placed among the resident *T. parviflora* stand during August when the beetles reached high populations and defoliated the *T. parviflora*, but the *F. salina* plants later recovered.

These open-field tests confirmed the cage-test results of Lewis *et al.* (2003a), Milbrath and DeLoach (2006a,b) and Herr *et al.* (unpublished data) that the female beetle searching for an ovipositional host plant is the most highly selective life stage. Adult plant selection for alighting/ feeding or larval development is less specific. These results suggest that, if athel grows within a saltcedar stand, adults will alight on athel, but females will deposit fewer eggs than on saltcedar, leading to smaller larval populations and less damage to athel than to saltcedar. In the absence of a choice, i.e. if athel is spatially isolated from saltcedar or occurs in the midst of a defoliated saltcedar stand, the beetles would be unlikely to establish populations on athel or to cause damage sufficient to interfere with its use as a shade or windbreak tree.

We met three times with Mexican scientists and officials, and in June 2007, they agreed not to oppose our releases along the Rio Grande of west Texas. Therefore, on 29 June 2007, we released the Crete beetles from seven overwintering cages on five private ranches. So far, increases in beetle populations have been slow and most sites have flooded, but the beetles have survived at most sites, and at three sites populations have increased to a few hundred or a few thousand near the cages, suggestive of establishment. In general, the establishment of the southern-adapted beetles in the field has had a lower rate of success and lower rates of increase and dispersal than that of the Fukang/Chilik ecotype released in the northern areas. However, damage to saltcedar still is sufficient to promise successful biological control.

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# Monitoring the rust fungus, *Puccinia jaceae* var. *solstitialis*, for biological control of yellow starthistle (*Centaurea solstitialis*)

**A.J. Fisher,[1] D.M. Woods,[2] L. Smith[1] and W.L. Bruckart[3]**

*Summary*

Yellow starthistle, *Centaurea solstitialis* L., is a noxious weed that infests more than 7 million ha of rangeland in California. The rust fungus, *Puccinia jaceae* Otth var. *solstitialis*, was first released as a classical biological control for yellow starthistle in California in 2003. In 2005, a research program was initiated to monitor the life cycle and spread of *P. jaceae solstitialis*. The rust was released at two sites representing different climatic zones, the coastal hills and Central Valley, in January 2005 and 2006. Releases resulted in infected plants at both sites in both years. Natural urediniospore (infective spore) reinfection occurred throughout yellow starthistle's growing season at the Central Valley site, but infection did not persist at the coastal hills site. *P. jaceae solstitialis* spread at least 100 m in 2005 at the Central Valley site but did not spread at the coastal hills site. The results of this study show that the spread of the rust is most concentrated in areas closest to release sites. Teliospores (dormant spores) were produced during plant senescence at both sites in 2005. Our results suggest that *P. jaceae solstitialis* is likely to establish and spread to new yellow starthistle populations in the Central Valley but not in the coastal hills, near Napa California.

***Keywords***: reinfection, establishment, spread.

## Introduction

Yellow starthistle (*Centaurea solstitialis* L., Asteraceae) is an invasive alien weed that infests more than 7 million ha of rangeland in California (Pitcairn *et al.*, 2006). Yellow starthistle displaces desirable plants in both natural and agricultural areas. Its spiny flowers deter feeding by grazing animals and lower the value of recreational lands (Sheley *et al.*, 1999). Yellow starthistle is a winter annual adapted to the mild wet winters and dry summers of the Mediterranean (Maddox, 1981). Seeds usually germinate soon after the beginning of fall rains, rosettes develop slowly during the winter and plants bolt in late spring and flower continuously until the plant senesces from lack of moisture. Scientists have introduced six insect biological control agents, all of which attack flower heads and destroy developing seeds (Turner *et al.*, 1995; Pitcairn *et al.*, 2004). However, these have not reduced yellow starthistle populations to acceptable levels (Balciunas and Villegas, 1999; Woods *et al.*, 2004).

In 2003, the rust fungus, *Puccinia jaceae* Otth var. *solstitialis*, was introduced to California for the biological control of yellow starthistle (Woods *et al.*, 2003). This is the first exotic plant pathogen to be approved for release for the classical biological control of a weed in the continental USA using the modern permitting process required by the Animal and Plant Health Inspection Service (APHIS; Bruckart *et al.*, 1999).

*P. jaceae* var. *solstitialis* is macrocyclic (its life cycle includes five spore stages), and it conducts its entire life cycle on yellow starthistle (Savile, 1970). Plants infected during the growing season produce pustules that release urediniospores. Urediniospores disperse aerially to infect other leaves and plants, resulting in multiple generations within a growing season that may progressively increase the incidence, intensity and spatial spread of infection. The latent period (time from infection to spore formation) ranges from 10 to 15 days

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at 25°C to 15°C, respectively (Bennet *et al.*, 1991). Many rust fungi produce teliospores, dormant spores, during plant senescence. Teliospores germinate in the presence of a host plant to produce basidiospores during the wet season. To complete the life cycle, basidiospores are thought to infect yellow starthistle seedlings to produce pycnia. Pycnia have been observed on yellow starthistle seedlings in California at a site where *P. jaceae solstitialis* was released the previous year (Fisher *et al.*, 2006). Pycnia produce aecia, which produce urediniospores to continue the infection cycle (Savile, 1970).

In 2005, a research program was initiated to monitor the life cycle and spread of *P. jaceae solstitialis* in California. The goals of this study were to: (1) monitor urediniospore natural reinfection over the yellow starthistle growing season, (2) monitor teliospore emergence and (3) monitor the spread of *P. jaceae solstitialis* at two release sites in California.

# Materials and methods

## Fungal isolate and site characteristics

*P. jaceae* var. *solstitialis* field isolate FDWSRU 84-71 was collected by S. S. Rosenthal in 1984 east of Yarhisar and Hafik (near Šivas), Turkey. This isolate was used for host-specificity testing and was the isolate released from quarantine (Bruckart, 1989; Bruckart *et al.*, 1999). Urediniospores for the present study were propagated at the California Department of Food and Agriculture, Sacramento, CA, using the methods described by Woods and Popescu (2004).

Permanent experimental plots were established in January 2005 and 2006 at sites near Napa and Woodland, CA. The Napa site is an ungrazed rangeland in the coastal hills near Napa, Napa County, 427-m elevation, dominated by yellow starthistle and exotic European annual grasses, and surrounded by oak and manzanita forest. The Woodland site is an ungrazed rangeland, near Woodland, Yolo County, 5-m elevation. It is dominated by exotic European annual grasses, surrounded by actively grazed rangeland and agriculture. The Woodland site is in the Central Valley, which has a hot summer, and the Napa site is in the coastal hills, which is cooler.

## Urediniospore natural reinfection over a single growing season

In 2005, six permanent 1 × 0.5 m plots marked by wooden stakes were installed at each site. In late January 2005, each plot was uniformly sprayed with 200 ml of deionized water containing 50 mg urediniospores and 0.15% Tween 20 (=100 mg spores/m$^2$) using plastic 250-ml finger pump spray bottles. This was sufficient to wet all plants in the plot to runoff. A portable rectangular wall of plastic sheeting was placed around each plot to prevent drift during application. Plots were evaluated 30, 60, 90 and 120 days after inoculations and scored either as positive or negative for disease symptoms.

In 2006, the experiment was repeated on a smaller scale, and six additional 0.5 × 0.5 m plots were installed. Plots were inoculated on the same dates, using the same methods, as in 2005.

## Teliospore emergence

In addition to the 12 plots above, a separate 1-m$^2$ plot was inoculated at both sites, with 200 ml of deionized water containing 50-mg spores and 0.15% Tween 20, to monitor teliospore emergence in 2005. Plots were reinoculated once a month from February to June, if needed, to maintain infection. Once a month, from March to September, five infected leaves were harvested from each site. In the lab, spores were scraped off leaves into 0.1% water agar, and the number of urediniospores and teliospores in a 200-spore sample from each leaf was counted using a compound microscope. The Proc GLM procedure was used to evaluate the effect of location (Napa or Woodland) on teliospore production. Analyses were carried out using SAS Institute software version 9.1 (SAS Institute, 2000).

## Monitoring *P. jaceae solstitialis* spread

In a separate study to develop an optimal strategy to release *P. jaceae solstitialis* (Fisher *et al.*, 2007), 9 g of urediniospores were used to inoculate yellow starthistle at the two sites described above. Plots of yellow starthistle were inoculated up to five times from January to June. At the first inoculation, plants were at the rosette stage, and during the last inoculation, plants were beginning to flower. To document spread, permanent 0.5 × 2 m plots were installed 20, 40, 60, 80 and 100 m from inoculated plots at both sites in May 2005, positioned along four compass directions. Because of yellow starthistle patchiness and site boundaries, there were a total of seven monitoring plots 20 m from release plots, five monitoring plots 40 and 60 m from release plots, four monitoring plots 80 m from release plots and six monitoring plots 100 m from release plots. In May 2005, we visually inspected plants within a metre on all four sides of each of the six plots inoculated in January to observe natural urediniospore spread. In June 2005, we visually inspected all monitoring plots 20, 40, 60, 80 and 100 m from inoculated plots and recorded the number of infected plants out of a maximum of 50 plants per plot. To determine if the rust fungus had spread beyond 100 m in July 2005, roadside yellow starthistle populations were inspected for *P. jaceae solstitialis* infection at the Central Valley site up to a distance of approximately 5 km.

## Results and discussion

*P. jaceae* var. *solstitialis* pustules emerged on yellow starthistle at both sites in 2005 and 2006 within 30 days after inoculations (Fig. 1). In the coastal hills (Napa site) in 2005, rust pustules were present in all plots after 60 days but declined to three of six plots after 90 days and then to two of six plots after 120 days. In 2006 at the Napa site, there was no natural reinfection at plots inoculated in 2005, and plots inoculated in 2006 had pustules only at 30 days after inoculation. All six plots in the Central Valley (Woodland site) contained infected plants at each sampling date in both years. It is not clear why *P. jaceae solstitialis* did not consistently persist in release plots at the Napa site. To identify factors that limit the success of the pathogen, it will be necessary to monitor local climate conditions. Bennett *et al.* (1991) estimated that optimal *P. jaceae solstitialis* infection occurs at 15–20°C with dew periods between 8 and 16 h. Data on dew periods at these locations would allow us to determine if and when these criteria are met in the field.

As the season progressed, temperatures rose and yellow starthistle senesced; *P. jaceae solstitialis* produced more teliospores (dormant spores that germinate the next winter) as a proportion of all spores. Production of teliospores gradually increased at both sites in August and September 2005 (Fig. 2). A higher percentage of spores sampled in the Central Valley were teliospores ($P < 0.001$) compared to the coastal hills.

The rust spread up to 100 m at the Woodland site in 2005 (Fig. 3). In May of 2005, all of the plots inoculated in January had pustules at least 1 m outside of plots at the Woodland site. By July, the rust had spread to one of the five monitoring plots 100 m from the nearest release plot. Of the monitoring plots, the highest percentage with rust, aside from the metre surrounding plots, occurred at 60 m (Fig. 3). It is not clear why a greater percentage of plots had rust at 60 m compared to 20 or 40 m. When we compare the percentage of

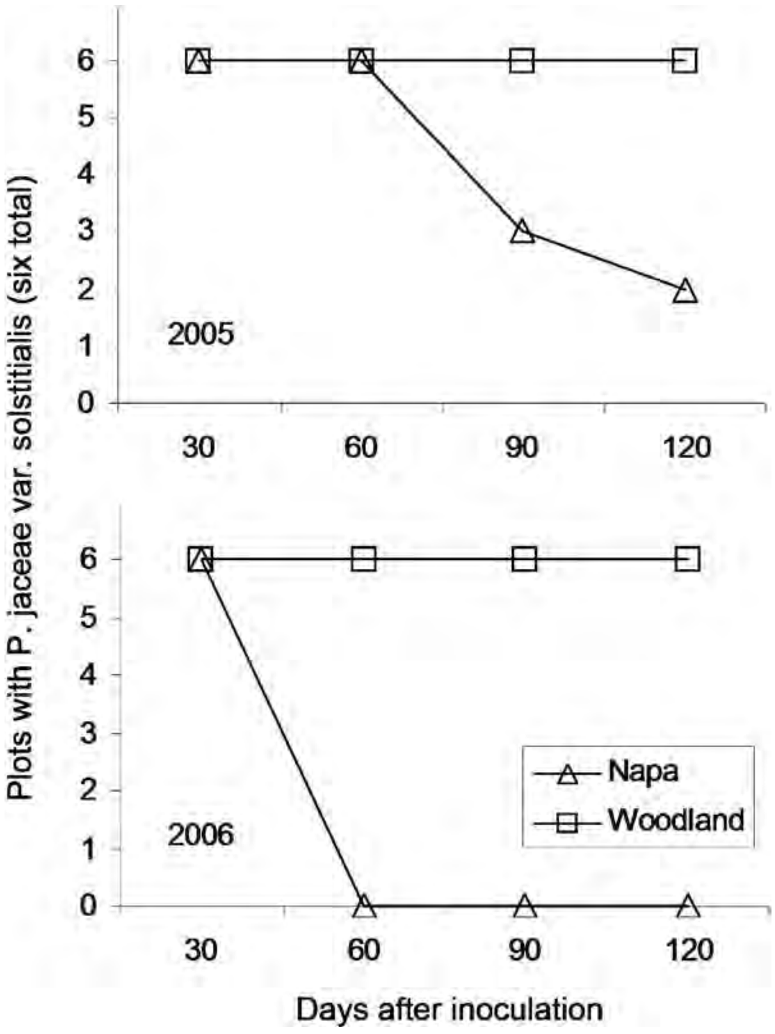


**Figure 1.** Occurance of *Puccinia jaceae* var. *solstitialis* pustules indicating recent infection of yellow starthistle plants in permanent plots that were inoculated in late January in 2005 and 2006.

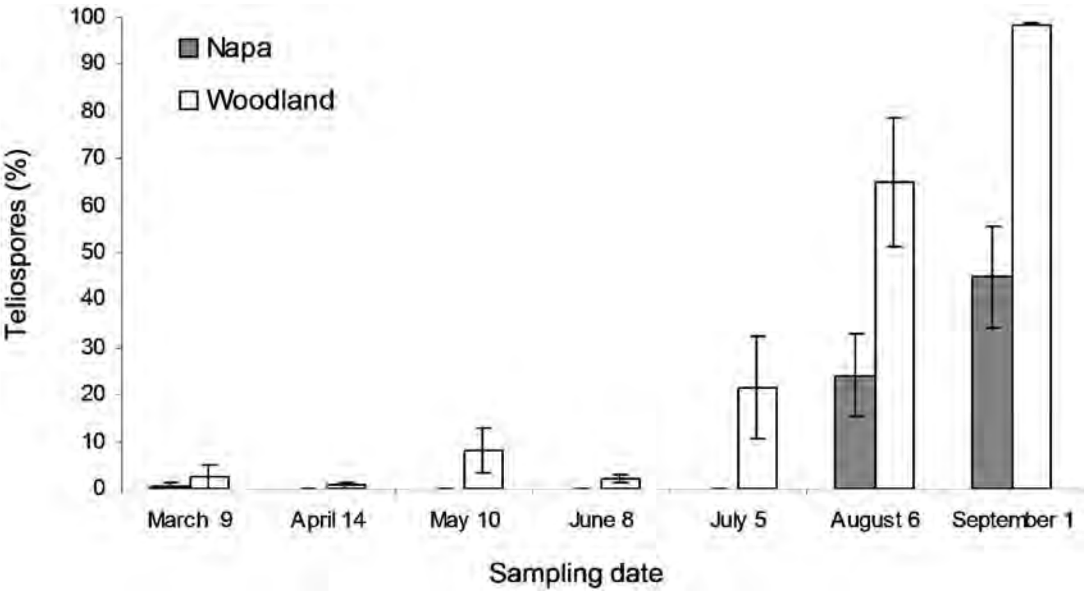


**Figure 2.** Natural production of *Puccinia jaceae* var. *solstitialis* teliospores (dormant spores), as a proportion of all spores (means ± SE), at the Napa and Woodland, CA, sites in 2005 after inoculations in release plots starting in January 2005.

plants in each plot with at least one pustule, 26% of the plants in plots 20 m from release plots were infected, compared with 2% in 40-m plots, 6% in 60-m plots, 2% in 80-m plots and 2% in 100-m plots. These data show that the spread of the rust is most concentrated in areas closest to the release plots. No rust fungus was found during visual inspection of roadside yellow starthistle populations surrounding the release site (up to 5 km away). Spread was modest compared to other successful biological control pathosystems, for example the white smut fungus, *Entyloma ageratinae* Barreto and Evans, released for mist flower, *Ageratina riparia* (Regel) King and Robinson, spread 80 km over water in 2 years (Barton *et al.*, 2007).

The year after plots were inoculated, *P. jaceae solstitialis* naturally reinfected yellow starthistle at the site in the Central Valley but not in the coastal hills (Fisher *et al.*, 2007). For *P. jaceae solstitialis* to reinfect yellow starthistle after a dormant season, it must produce teliospores that persist and germinate in the winter to complete the life cycle. Teliospores were observed in the summer, and pycnia were observed in release plots

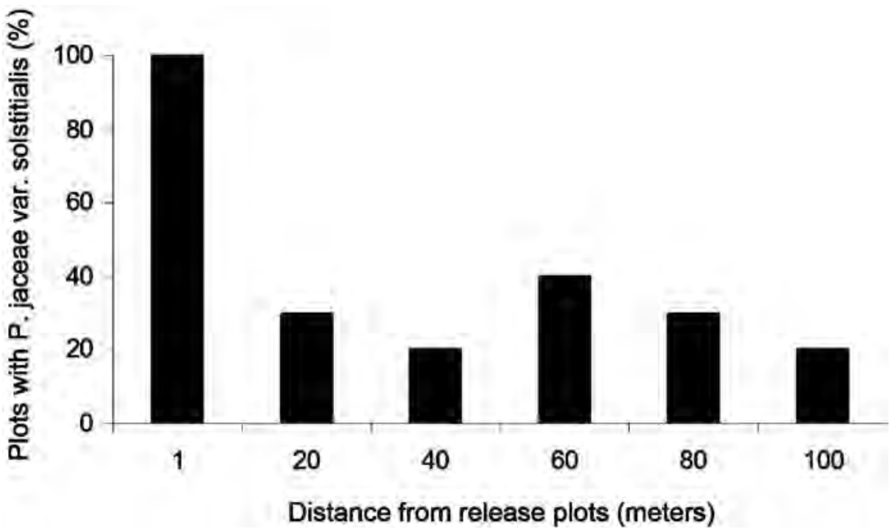


**Figure 3.** Spatial spread of *Puccinia jaceae* var. *solstitialis*, as represented by pustules on yellow starthistle plants during one growing season in Woodland, CA, in 2005.

the following February (Fisher *et al.*, 2006). Soon after pycnia were observed, in March, urediniospores were identified in the 2005 release plots.

In general, the rust fungus was more productive in the Central Valley (Woodland site) than the coastal hills (Napa site), with higher rates of reinfection, both within a single growing season and after a dormant season. In addition, *P. jaceae solstitialis* spread at least 100 m during the first year at the Central Valley site. In the coastal hills, yellow starthistle exhibited disease symptoms soon after inoculation, but infection decreased over time and did not return the following year. These results suggest that this isolate of *P. jaceae solstitialis* should establish well in California's Central Valley but not at sites with climate conditions similar to the coastal hills.

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# Is ragwort flea beetle (*Longitarsus jacobeae*) performance reduced by high rainfall on the West Coast, South Island, New Zealand?

**A.H. Gourlay, S.V. Fowler and G. Rattray**

*Summary*

The New Zealand biological control programme against ragwort, *Senecio jacobeae* L., began in the 1920s, and four biological control insects have been released. The ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera, Chrysomelidae), was introduced from Oregon into the field in 1983. The beetle has established and has had a major impact on ragwort plant populations in many areas of New Zealand, but not on the West Coast of the South Island. Field studies looked at sites where the beetle had established to determine why it had not reduced ragwort populations. The abundance of ragwort plants (13/m$^2$) and of flea beetles (three per rosette) was recorded at five sites. The biology of flea beetles and ragwort plants at each site were also recorded and rainfall data were collected. The inability of the ragwort flea beetle to reduce ragwort populations in some areas in New Zealand may be due to the high density and rapid growth of ragwort plants, possibly due to higher rainfall, and this may also have a negative effect on flea beetle populations. Numbers of flea beetles per plant was lower on the West Coast (four per rosette) than East Coast sites (ten per rosette). The density of ragwort plants was higher on the West Coast (13/m$^2$) than the East Coast (4/m$^2$).

***Keywords:*** ragwort flea beatle, *Longitarsus jacobaeae*, rainfall.

## Introduction

The biological control programme against ragwort, *Senecio jacobaeae* L. (Asteraceae), began in the 1920s and was one of the first such programmes implemented in New Zealand. Four biological control insects have been released: two seed-feeding flies, *Botanophila seneciella* (Meade) and *Botanophila jacobaeae* (Hardy), both from England, one foliage-feeding moth, *Tyria jacobaeae* (L.), from England and a root-feeding flea beetle, *Longitarsus jacobaeae* (Waterhouse), from Oregon. Only one of the seed-feeding flies, *B. jacobaeae*, has established and only in the central North Island. The foliage-feeding moth has established sporadically throughout both islands, reducing plant seeding and height, but it has had little impact on plant populations. However, the flea beetle has established in many areas of New Zealand and has had a major impact on ragwort plant populations. Reductions of 90–100% in plant density have been recorded at many sites in 2 to 10 years after initial release and establishment of the flea beetle (Hayes, 1996). There are two main exceptions to the successful establishment of the flea beetle and the reduction in ragwort populations: on the West Coast and southern regions of the South Island and in western parts of the North Island (Hayes, 1998).

Ragwort is a biennial or perennial herb. Early in winter, plants develop into a leafy rosette 2–5 cm high and up to 15 cm in diameter. Daisy-like bright yellow flowers bloom from November to January. Stems are reddish purple and can grow up to 60 cm. Each plant can produce up to 250,000 seeds a year; these can lie dormant in the soil for up to 16 years. Ragwort seeds are dispersed by wind and water, on vehicles, machinery, clothing and in hay and chaff. The plant reproduces through seed and vegetatively from cut root fragments (Meander Valley Weed Strategy, 1999).

Field experiments were conducted at 11 sites in New Zealand, where, in paired plots, ragwort plants

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were sprayed with Halmark® to remove flea beetles and compared with ragwort on unsprayed plots. The density of ragwort plants and flea beetles in each plot was measured over 2 years at each site. In some cases, these plots have been monitored for a further 2 years, although spraying was discontinued in 2003. The data showed that the flea beetle significantly reduced ragwort plant density within 1 or 2 years. Once established, the flea beetle continued to keep ragwort plant densities at low levels (P. McGregor, 2001, unpublished results).

This study was initiated because it was not clear why this success is not reflected at release sites in western areas of New Zealand. This paper describes the study results.

## Methods

Five sites (Table 1) were chosen, where the flea beetle was present and because they represented diverse geographical and climatic areas throughout the West Coast region.

At each site, 20 juvenile (rosette), 20 mature (bolting) and 20 multi-crown ragwort plants were selected, and adult flea beetles were collected from them using a leaf sucker. Beetle numbers were recorded per plant. At 20 randomly selected locations at each site, we counted the total number of juvenile (rosette), mature (bolting) and multi-crown ragwort plants per 0.25 $m^2$ quadrat.

At each site, we measured and recorded the rosette diameters of 50 juvenile plants, the basal diameters of 50 mature plants and the total basal diameters of 50 multi-crown plants to determine the total dry biomass of ragwort per size class of plant per quadrat. Where multi-crown plants occurred, 30 plants, including roots, were collected to calculate the total dry biomass of multi-crown plants.

Monthly rainfall data were recorded for each site, and a maximum daily rainfall event was also retrieved from NIWA's National Climate Database (via the Cli-Flo Web Access Service). Data for daily temperatures were not available for these sites.

Twenty beetles from each site were placed in vials of 70% alcohol and later dissected in the laboratory to determine the sex ratio and age by looking at the state of the wing muscles: Newly emerged adults have wing muscles, but older beetles lose their wing muscles after summer aestivation and mating.

Landowners were asked if any weed control or other relevant treatments had taken place on the pasture. During sampling, we noted the weather and recorded an estimate of soil moisture content. Other features

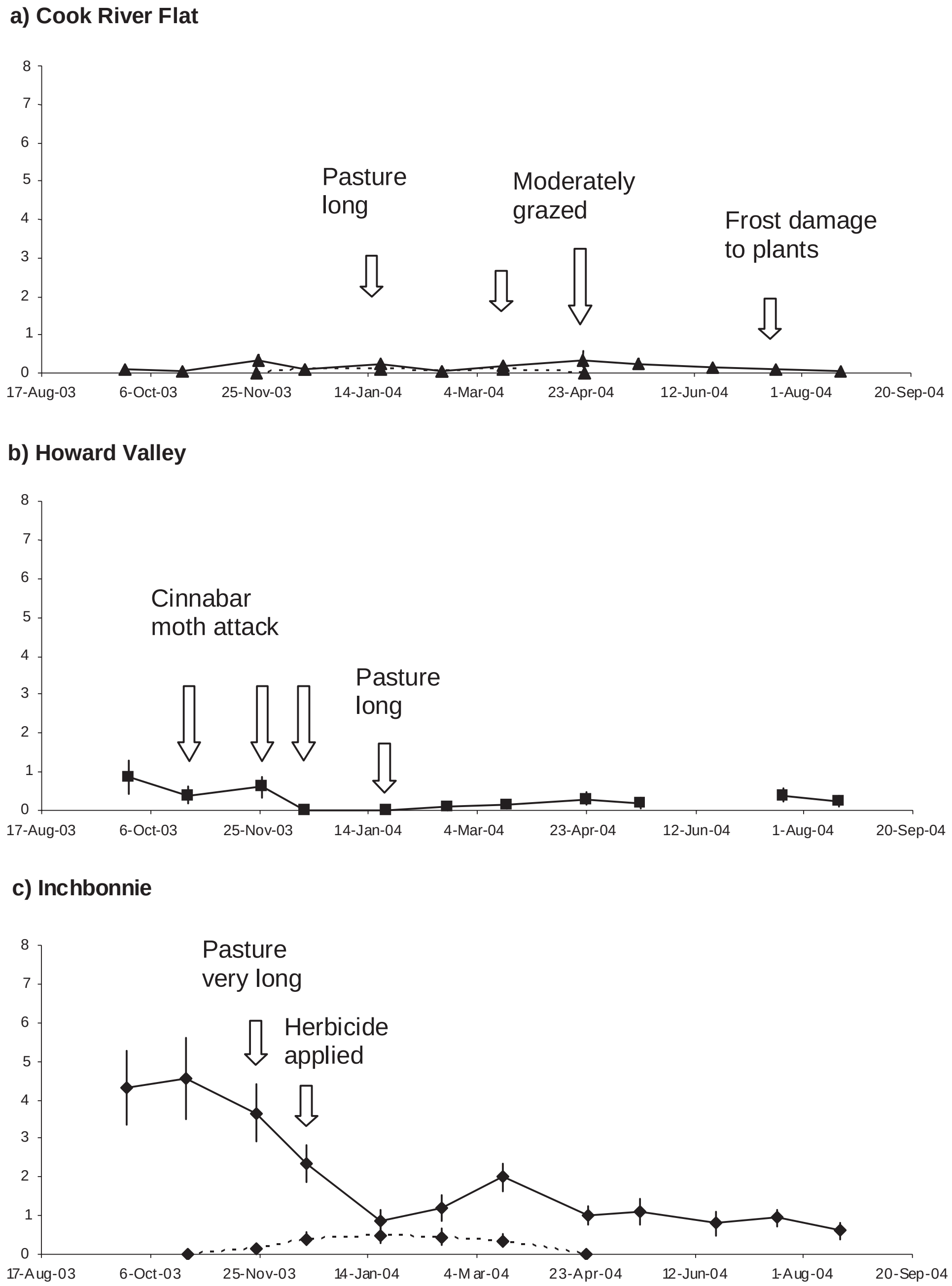
**Table 1.** Maximum ragwort density and maximum beetle numbers per rosette at other New Zealand sites (P. McGregor, 2001, unpublished data).

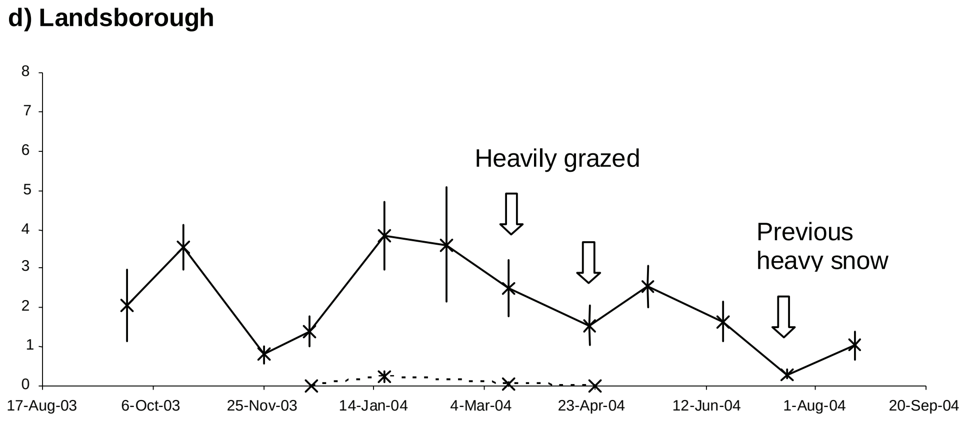
| Site | Max. ragwort density | Max. beetle numbers | Comments |
|---|---|---|---|
| Orere Farms (Auckland) | 2.3 | 50 | Dramatic drop in plant numbers in water-sprayed plots compared with plots treated with Hallmark insecticide |
| Southland | 1.8 | 25 | Fluctuating numbers of ragwort plants but no effects of Hallmark insecticide despite large beetle numbers |
| H/I BoP | 1.3 | 15 | Large drop in plant numbers before Hallmark insecticide used |
| Wellington | 1.6 | 11.2 | Large drop in plant numbers before Hallmark insecticide used |
| Manawatu-Wanganui | 0.4 | 10.6 | Ragwort numbers very low at start, but significant increase in plant density when Hallmark insecticide applied |
| Sisam, BoP | 3.5 | 8 | Large drop in plant numbers before Hallmark insecticide used |
| McCann BoP | 1.1 | 4 | No significant changes in plant numbers, over time or in presence/absence of Hallmark insecticide |
| Alcove Properties | 2.4 | 3 | Complex patterns perhaps due to Kikuyu grass competition |
| Nett. BoP | 1.4 | 1 | No significant changes in plant numbers, over time or in the presence/absence of Hallmark insecticide |
| Taranaki | 1.3 | 1 | No significant changes in plant numbers, over time or in presence/absence of Hallmark insecticide |
| West Coast | 13 | 1 | No significant changes in plant numbers, over time |

of the pasture were also recorded, e.g. pasture length, how heavily grazed the pasture was, snow fall, extreme weather events and whether or not it had been sprayed. To determine the range where impacts on individual plants might be expected, we compared the West Coast with sites elsewhere in New Zealand. Information was extracted from impact experiments carried out by regional council staff in collaboration with Landcare Research (P. McGregor, 2001, unpublished data) on the density of ragwort plants and the mean number of flea beetles recorded per rosette (Table 1). With the exception of the Southland site, all sites above the dotted line in Table 1 (which had maximum beetle numbers of eight or more per rosette) showed changes in plant density consistent with successful suppression by the flea beetle. Conversely, all the sites with maximum beetle numbers per rosette of four or less showed no indication of any effective suppression of plant density by ragwort flea beetle.

## Results and discussion

Some variations in ragwort densities could have been caused by weather. For example, the monthly low in the density of juvenile plants in July 2004 at Landsborough Valley (Fig. 1) might have been caused by recent heavy snow. Some frost damage to ragwort plants was also noted in July 2004 at Cook River Flat (Fig. 1). Otherwise, there was no apparent effect of abiotic factors on the abundance of ragwort plants or our ability





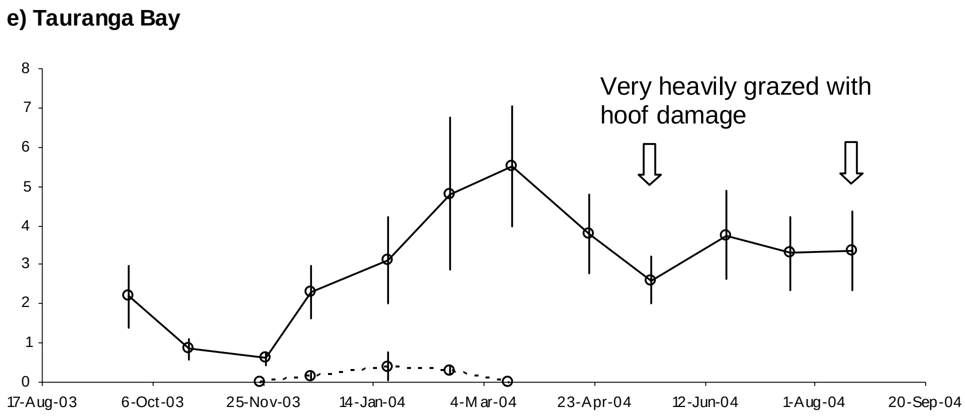




**Figure 1.** Number of ragwort plants per 0.25-m$^2$ quadrat during the study period. Plants were recorded as juvenile (rosette stage) shown as solid lines or as adults with flowering stem(s) shown as dotted lines. Each point is a mean of 20 quadrats per date per site; bars are ±1 SEM.

to sample them (except when sampling was prevented by snow in July 2004 at Howard Valley). None of the rainfall measurements (daily peak per month, monthly total and total for year) were correlated with ragwort density. Monthly rainfall data are summarized in Table 2. Flea beetles were found at three sites only. They were Landsborough Valley, Tauranga Bay and Howard Valley.

Plant density and flea beetle density were not related at any of the sites sampled (Fig. 2). Table 1 suggests that the impacts of the flea beetle on ragwort plant density are only likely when beetle numbers per rosette exceed four (P. McGregor, 2001, unpublished data). The highest numbers of beetles recorded were from multi-crowned plants (mean peak of four or more per

**Table 2.** Monthly rainfall data (mm) for the five study sites.

| Site/month | Tauranga Bay | Landsborough Pleasant Flat | Cook River Flat | Porika Hills Howard Valley | Red View Farm |
|---|---|---|---|---|---|
| September 2003 | 199 | 630 | 588 | 22 | (no record) |
| October 2003 | 181 | 200 | 422 | 124 | 310 |
| November 2003 | 144 | 410 | 484 | 127 | 412 |
| December 2003 | 164 | 318 | 476 | 108 | 374 |
| January 2004 | 265 | 511 | 728 | 118 | 585 |
| February 2004 | 234 | 493 | 783 | 260 | 746 |
| March 2004 | 86 | 382 | 491 | 38 | 484 |
| April 2004 | 105 | 76 | 122 | 28 | 136 |
| May 2004 | 230 | 460 | 606 | 158 | 400 |
| June 2004 | 323 | 555 | 618 | (no record) | 677 |
| July 2004 | 133 | 158 | 277 | 86 | 190 |
| August 2004 | 201 | 430 | 446 | 178 | 563 |
| Yearly total | 2265 | 4623 | 6041 | 1247 | 4877 |

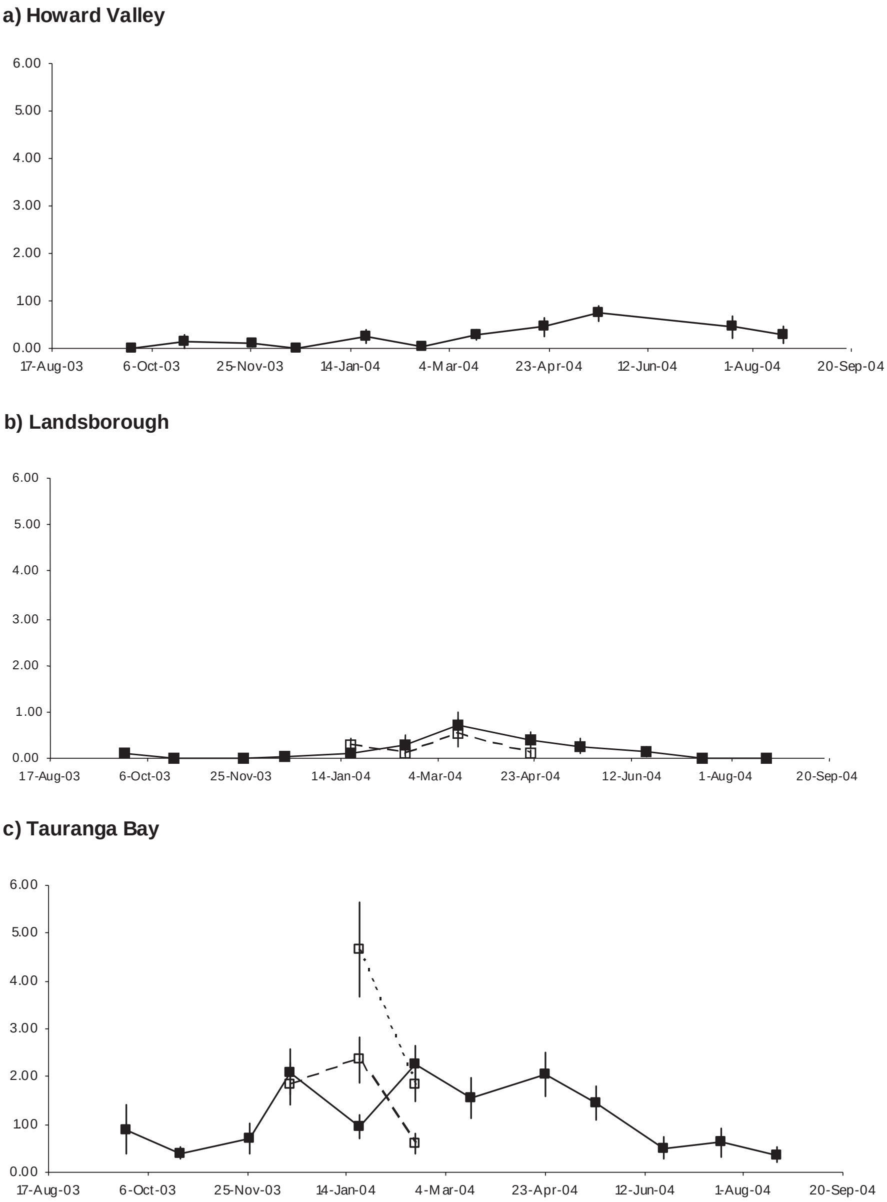



**Figure 2.** Mean number (±SEM) of beetles per plant at the three sites where they were found. Samples were from 20 plants except on a few occasions when fewer than 20 could be located. Three plant types were sampled: juveniles (rosettes), solid lines; adults (one flowering stem), dashed lines; multi-stemmed adults dotted lines (these only occurred at Tauranga Bay). In 2003–2004, no ragwort plants matured at the Howard Valley site.

plant, Tauranga Bay, January 2004). The highest number of beetles recorded from a single rosette was ten beetles at Tauranga Bay on 25 Sept. 2003, which was still relatively low compared with the numbers in Table 1 (P. McGregor, 2001, unpublished data).

In addition, numbers of beetles on single-stemmed adult plants peaked on this date, coinciding with a marked decrease in the number of beetles collected from juvenile plants. This suggests that multi-crowned plants attracted beetles from the smaller single-stemmed and rosette plants. However, previous studies showed that, although adult beetles can be abundant on large, multi-crowned plants, the number of larvae inside the roots is low per gram dry weight compared with rosette plants (James *et al.*, 1992).

In general, beetle numbers at the three sites were lowest (less than one beetle per plant) in winter/spring and increased over summer/autumn to four per plant (Fig. 2). The numbers of beetles collected were not related to monthly rainfall, peak daily rainfall (per month) or weather conditions. However, there was a trend suggesting that beetle densities were lower at sites with higher annual rainfall (Fig. 3).

Dissections showed that new adults were present from September to March, peaking in December (when no sexually mature adults were collected at any site).

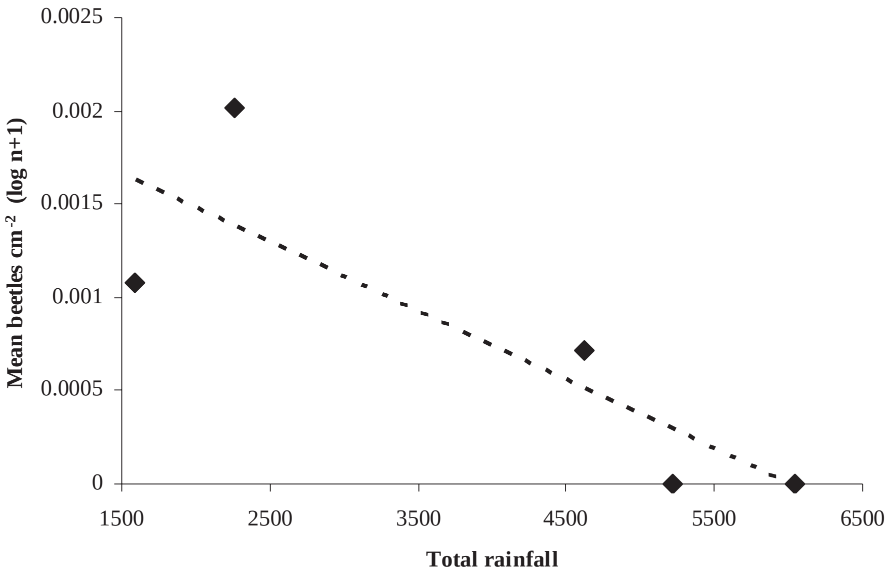



**Figure 3.** Mean number of ragwort flea beetles collected per unit area (cm$^2$) of juvenile plants plotted against the total recorded rainfall for the study year at each of the five sites. The relationship approaches statistical significance ($P$ = 0.07, $r^2$ = 0.61, $y$ = (−3.7 × 10) −7$x$ + 0.0022, $F_{(1,3)}$ = 7.20).

The pattern of wing muscle appearance in adult beetles on the West Coast shows that the flea beetle has a single generation per year, that the females live for about a year and that they are capable of laying eggs throughout the rest of the winter and after spring. The ratio of males to females in each sample was approximately 1:1 at the Howard Valley site and did not differ significantly over the year. However, at Tauranga Bay and Pleasant Flat Valley twice as many males as females were collected during the year (Fig. 4).

The mean beetle numbers per rosette in our West Coast study never exceeded three per rosette (Table 1). The numbers per individual rosette plant only exceeded four at one site, Tauranga Bay, and this threshold was exceeded in only seven of the 12 monthly sampling dates, although only in 15 rosettes out of the total of 240 sampled during the study year. When rainfall for each site, where beetles were found, was overlaid onto the graphs of beetle and plant density, these events appeared not related (Fig. 3). Sites where beetles were not detected were not used in the analysis. Thus, although the high rainfall on the West Coast may affect beetle density, this remains unproven.

Ragwort, typically a biennial plant, will behave as a perennial if the flowering stalk is cut, grazed, mowed, trampled or mechanically injured while flowering

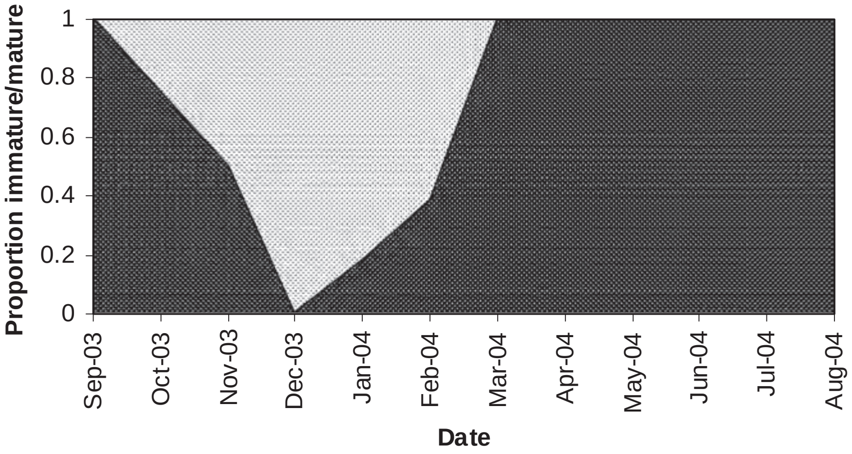



**Figure 4.** Proportion of dissected adult female beetles that were mature (dark areas) and immature (lighter areas) during the study period.

(McEvoy, 1984). After such damage, the plant can regenerate from crown buds, root fragments or intact roots. When flowers are removed before they set seed, the plant can re-flower later the same season. Defoliated rosettes will continue to grow for several years as vegetative perennials. The heavy-stocking regime at the Tauranga Bay site appears to have allowed perennial plants to survive and set much seed. It may also have created gaps in the pasture and allowed substantial seedling recruitment throughout the year (Fig. 1).

The number of beetles per plant was lower at the West Coast sites (a maximum of ten but an average of one beetle per plant) than some East Coast sites where ragwort has been controlled by the flea beetle (an average of 40 beetles per plant at one site but an average range of eight to 50 beetles per plant; P. McGregor, 2001, unpublished data).

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# Host-range investigations of potential biological control agents of alien invasive hawkweeds (*Hieracium* spp.) in the USA and Canada: an overview

**G. Grosskopf,[1] L.M. Wilson[2] and J.L. Littlefield[3]**

*Summary*

Several European *Hieracium* species, e.g. *Hieracium caespitosum* Dumort. and *Hieracium aurantiacum* L., are noxious weeds in North America. A project for the biological control of alien invasive hawkweeds has therefore been initiated in 2000. Five European insect species investigated before their release in New Zealand and two additional gall wasps have been tested on North American test plants. The stolon-tip galling cynipid, *Aulacidea subterminalis* Niblett (Hym., Cynipidae) proved to be the most specific candidate attacking four *Hieracium* spp. in the subgenus *Pilosella*. *Aulacidea hieracii* (L.), a gall wasp reared from *Hieracium procerum* Fries (subgenus *Pilosella*) and *Hieracium robustum* Fries (subgenus *Hieracium*), which severely galls the flower stalks, did not attack any of the target weeds. Another gall wasp, *Aulacidea pilosellae* (Kieffer), galling the midrib of leaves, stolons and flower stalks, attacked two native North American hawkweed species under no-choice conditions but none of the natives exposed in open-field tests. As a negative effect on the target weeds has not yet been shown, host-range investigations are postponed. Preliminary tests with *Oxyptilus pilosellae* Zeller (Lep., Pterophoridae) were stopped due to attack of non-target species. *Macrolabis pilosellae* (Binnie) (Dipt., Cecidomyiidae), a multivoltine gall midge galling the rosette centre, flower heads and stolon tips, can develop on most native North American *Hieracium* spp. As attack occurred also in field cages and in the field, this agent was removed from the list of potential agents. The root-feeding hoverfly, *Cheilosia urbana* (Meigen) (Dipt., Syrphidae), and the rosette-feeding hoverfly, *Cheilosia psilophthalma* (Becker), develop on seven and at least two native hawkweed species, respectively, in no-choice larval transfer tests. However, under open-field conditions, attack rates of *C. urbana* on native *Hieracium* spp. are much lower than on *H. caespitosum*. Further experiments are planned to explore the level of *C. urbana* attack in the field. Neither test nor control plants were attacked by *C. psilophthalma* in open-field tests in 2005 and 2006. Therefore, host-range tests with *C. psilophthalma* are progressing slowly.

***Keywords***: host specificity, non-target feeding, *Cheilosia urbana*, *Cheilosia psilophthalma*, *Aulacidea pilosellae*, *Aulacidea hieracii*, *Aulacidea subterminalis*, *Macrolabis pilosellae*.

## Introduction

Several *Hieracium* spp. (Asteraceae, Lactuceae) of Eurasian origin have become troublesome weeds in pastures, nature reserves, roadsides, and in deforested areas in New Zealand (Syrett and Smith, 1998) and North America (Wilson *et al.*, 1997). The most invasive hawkweed species in North America are species within the subgenus *Pilosella*, which have a high rate of reproduction and dispersal due to high seed output and asexual propagation, e.g. *Hieracium caespitosum* Dumort. and *Hieracium aurantiacum* L. (Wilson and Callihan, 1999). Traditional management efforts, e.g. fertilizer and herbicide application, are not only costly but are also problematic in remote areas and nature reserves or other sensitive sites. A biological control project was therefore initiated in New Zealand in 1993

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(Syrett and Smith, 1998). Based on a literature review and initial field surveys, five European insect species attacking different plant parts of mouse-ear hawkweed, *Hieracium pilosella* L., were chosen for further investigation for New Zealand: the plume moth, *Oxyptilus pilosellae* Zeller, the gall midge, *Macrolabis pilosellae* (Binnie), the gall wasp, *Aulacidea subterminalis* Niblett, and the hoverfly species, *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker) (Syrett *et al.*, 1999; Grosskopf, 2006). Host-specificity tests indicated that these five insect species are at least genus-specific and therefore sufficiently host-specific for release in New Zealand (Syrett *et al.*, 1999; Grosskopf, 2006) where all *Hieracium* spp. are naturalized (Webb *et al.*, 1988). In contrast, approximately 29 *Hieracium* spp. in the subgenera *Chionoracium* and *Hieracium* are indigenous to North America (Strother, 2006). Therefore, to predict the potential host range of these biological control agents, native North American *Hieracium* spp. were tested with all of the above-mentioned insect species. *O. pilosellae* was removed from the list of potential biological control agents at an early stage due to difficulties in rearing the moth and its lack of specificity, and the results are therefore not presented. A second gall wasp, *Aulacidea pilosellae* (Kieffer), was collected from several target weeds in Germany, Poland and the Czech Republic, e.g. *H. aurantiacum* and *H. caespitosum*, and thus included in the list of potential agents (Grosskopf *et al.*, 2004a). A third cynipid, *Aulacidea hieracii* (L.), was collected from *Hieracium procerum* Fries (subgenus *Pilosella*) and *Hieracium robustum* Fries plants (subgenus *Hieracium*) in the Ukraine but repeatedly failed to produce galls on any of the target weeds (Grosskopf *et al.*, 2004a,b). This paper provides an overview of the host specificity and the current status of five potential biological control agents of invasive hawkweeds in North America, i.e. *A. subterminalis*, *A. pilosellae*, *M. pilosellae*, *C. urbana* and *C. psilophthalma*.

## Potential biological control agents

### *C. urbana* (Diptera, Syrphidae)

Females of *C. urbana* oviposit into the leaf axils of *Hieracium* spp., and the neonate larvae move into the soil to feed externally on the roots, creating small holes resulting in reduced above-ground biomass (Grosskopf, 2005). In Europe, mature larvae pupate in late September/October and overwinter within the soil, very close to the surface.

### *C. psilophthalma* (Diptera, Syrphidae)

Adults of this univoltine hoverfly emerge in April and May. Females oviposit into the leaf axils of hawkweed plants and the larvae feed on the above-ground plant parts, i.e. rosette centre, leaf axils and stolon tips (Grosskopf, 2005). The larvae are mobile and can migrate among plants as host quality decreases. Mature larvae pupate on the soil surface in late September and October. *C. psilophthalma* and the root-feeding hoverfly, *C. urbana*, often co-occur and have a very similar phenology (Grosskopf, 2005).

### *M. pilosellae* (Diptera, Cecidomyiidae)

This multivoltine gall midge deforms stolon tips, flower heads and rosette centres. Females oviposit in leaf axils close to the meristematic tissue. Larval feeding prevents the unfolding of the leaves, and *M. pilosellae* larvae live gregariously in-between them. Mature larvae move into the soil where they spin a cocoon in which they pupate. The gall midge has three generations at Delémont, Switzerland, and overwinters in the larval stage. Galled plants have shorter stolons and fewer flower heads than uninfested plants (Grosskopf, 2006). According to the literature, the host range of this gall midge is restricted to *Hieracium* spp. in the subgenus *Pilosella* (Buhr, 1964).

### *A. subterminalis* (Hymenoptera, Cynipidae)

This univoltine gall wasp induces multi-chambered galls in the tips of elongating stolons. The larvae overwinter within the galls, pupate in spring and adults emerge in May and June. Adults exhibit thelytoky. In host-range tests carried out for New Zealand, only two *Hieracium* spp. out of nine, i.e. *H. aurantiacum* and *H. pilosella*, were attacked (Syrett *et al.*, 1999), indicating a narrow host range.

### *A. pilosellae* (Hymenoptera, Cynipidae)

*A. pilosellae* is a small, uni- to bivoltine gall wasp, which induces galls on stolons, midrib of leaves and flower stalks (Buhr, 1964). Thus far, we have not been able to demonstrate significant impact of this insect on plant growth in garden studies (Grosskopf *et al.*, 2007).

## Materials and methods

### Test plant list

A test plant list was compiled by L. Wilson and J. Birdsall (2001, unpublished results) based on the phylogenetic approach proposed by Wapshere (1974, 1989). Emphasis was placed on native North American *Hieracium* species in the subgenera *Hieracium* and *Chionoracium* and target weeds in the subgenus *Pilosella*. Other plant species belonging to the closely related subtribes Crepidinae, Lactucinae, Microseridinae and Stephanomeriinae were also tested but were not attacked.

## Host-range tests

All tests were carried out at the CABI Europe-Switzerland Centre at Delémont, with the exception of *A. subterminalis* testing, which was conducted in quarantine facilities at Montana State University, Bozeman, MT, USA. As all insects are of Central European origin and known to have a narrow host range, open-field and field-cage tests could be carried out without restrictions at Delémont.

## No-choice tests

No-choice larval transfer tests were carried out with *C. urbana* and *C. psilophthalma*. Seven neonate larvae were transferred into the leaf axils of potted test plants. All plants were individually covered with gauze bags and embedded in sawdust in a garden bed. All pots were checked for immature stages from the middle of September onwards. In the case of *C. urbana*, the soil was sieved and checked for larvae and puparia. In tests carried out with *C. psilophthalma*, only the upper 5 cm of the soil and the above-ground plant parts were checked for immature stages.

As *M. pilosellae* adults are short-lived and hard to relocate, three males and three females were released onto potted test plants covered with gauze bags and were left on the plants during their entire life span. *A. subterminalis* was tested in sequential no-choice tests. Three females were transferred onto a caged test plant for 3 days, and transferred onto a different test plant afterwards. In contrast, due to their small size, *A. pilosellae* adults were not retrieved from the plants. Two females and two males of *A. pilosellae* were placed onto potted test plants covered with gauze bags. The number of galls was recorded on each test plant, at the earliest 4 to 6 weeks after exposure to the gall midge or gall wasps.

## Multiple-choice tests

Potted *Hieracium* plants, i.e. target weeds and test plants, were exposed to naturally occurring *Cheilosia* females in garden beds at Delémont in 2005 and 2006. In 2005, control plants (*H. caespitosum*) and test plants were exposed simultaneously. All plants were checked at the end of the summer for mature hoverfly larvae of *C. urbana* and *C. psilophthalma*. However, as numerous test plants died before evaluation of the tests in 2005, a two-phase open-field oviposition test was carried out in 2006. In the first phase, test and control plants (*H. caespitosum* and *H. aurantiacum*) were exposed simultaneously, while in the second phase, only native North American hawkweed species were exposed. The number of eggs on the different plants was recorded. As *C. urbana* and *C. psilophthalma* eggs cannot be distinguished, eggs were kept in separate Petri dishes for hatch, and freshly hatched larvae were transferred onto *H. aurantiacum* plants to determine the syrphid species at the pupal stage (Grosskopf *et al.*, 2007). Larvae of *C. urbana* and *C. psilophthalma* have high survival rates on *H. aurantiacum*. The gall midge *M. pilosellae* was tested in three field cages measuring 2 · 2 · 1.6 m. In two cages, *H. caespitosum* and test plants were exposed simultaneously to the gall midges and, in the third one, test plants only. *M. pilosellae* adults emerged from rearing pots placed into the field cages. *A. pilosellae* adults were released onto open-field plots previously used for host-range testing of *Cheilosia* (see above). Plants were checked for galls 6 to 8 weeks after release of the adults.

# Results and discussion

As none of the insects tested developed on any plant species outside the genus *Hieracium*, we only focus on the presentation of the hawkweeds tested. The testing programme was impeded by the difficulty in rearing *Hieracium* spp. in the subgenus *Chionoracium* resulting in numerous invalid replicates. Therefore, not all *Hieracium* spp. could be tested in sufficient replicates.

Of the five insect species presented in this paper, the cynipid, *A. subterminalis*, is by far the most specific potential biological control agent attacking four *Hieracium* spp. in the subgenus *Pilosella*, i.e. *H. pilosella*, *Hieracium flagellare* Willd., *H. aurantiacum* and *Hieracium floribundum* Wimmer & Grab. (Table 1). Its narrow host range is due to the fact that *A. subterminalis* females oviposit into the stolon tips, which are exclusively produced by *Hieracium* spp. in the subgenus *Pilosella*, whereas native North American hawkweed species never produce stolons for vegetative propagation. Insects that exclusively or mainly attack stolons are therefore given priority and should result in a lower risk of non-target effects. Although *H. caespitosum* plants from Idaho also produce stolons, they were not galled by *A. subterminalis*. This gall wasp appears to be sufficiently host specific for introduction into North America.

*Hieracium scouleri* and *Hieracium bolanderi*, both native North American hawkweed species, were utilized as hosts in no-choice tests carried out with *A. pilosellae* and adults emerged from *H. scouleri* galls. However, the gall wasp has not galled any of the indigenous *Hieracium* spp. exposed in open-field tests (Table 2) conducted to date. Although *A. pilosellae* seems to have a narrow field host range, screening tests are postponed since a negative impact on the target weeds has not yet been shown (Grosskopf *et al.*, 2007). Further impact experiments with this cynipid are being carried out in 2007.

Although gall-inducing insects are generally known to have a restricted host range (Shorthouse and Watson, 1976), the gall midge *M. pilosellae* does not show a sufficient level of specificity for field release in North America. *M. pilosellae* galls 12 indigenous

**Table 1.** No-choice tests with five potential biological control candidates.

| Insect species | *Cheilosia urbana* | *Cheilosia psilophthalma* | *Macrolabis pilosellae* | *Aulacidea subterminalis* | *Aulacidea pilosellae* |
|---|---|---|---|---|---|
| | % larvae retrieved | | % plants galled | | |
| Subgenus *Pilosella* | | | | | |
| *Hieracium aurantiacum* L. | 60.7 (*n* = 8) | 52.4 (*n* = 15) | 50.0 (*n* = 6) | 38.6 (*n* = 44) | 55.6 (*n* = 9) |
| *Hieracium caespitosum* Dumort. | 25.6 (*n* = 73) | 24.9 (*n* = 59) | 83.9 (*n* = 62) | 0 (*n* = 3) | 100 (*n* = 21) |
| *Hieracium flagellare* Willd. | | | | 50.0 (*n* = 16) | |
| *Hieracium floribundum* Wimm. et Grab. | 25.4 (*n* = 9) | 10.0 (*n* = 10) | 60.0 (*n* = 15) | 52.9 (*n* = 17) | |
| *Hieracium glomeratum* Froel. | 58.7 (*n* = 9) | 52.9 (*n* = 10) | 44.4 (*n* = 9) | 0 (*n* = 7) | 100 (*n* = 4) |
| *Hieracium pilosella* L. | 27.4 (*n* = 12) | 15.9 (*n* = 9) | 83.3 (*n* = 12) | 54.3 (*n* = 46) | |
| *Hieracium piloselloides* Vill. | 44.4 (*n* = 9) | 3.2 (*n* = 9) | 53.3 (*n* = 15) | 0 (*n* = 11) | 83.3 (*n* = 6) |
| Subgenus *Hieracium* | | | | | |
| *Hieracium canadense* Michx.[a] | 16.7 (*n* = 12) | 0 (*n* = 12) | 14.3 (*n* = 21) | 0 (*n* = 15) | 0 (*n* = 14) |
| *Hieracium umbellatum* L.[a] | 4.8 (*n* = 12) | 0 (*n* = 9) | 20.0 (*n* = 30) | 0 (*n* = 13) | 0 (*n* = 16) |
| Subgenus *Chionoracium* | | | | | |
| *Hieracium albiflorum* Hook.[a] | 0 (*n* = 3) | 9.5 (*n* = 3) | 5.9 (*n* = 17) | 0 (*n* = 18) | 0 (*n* = 5) |
| *Hieracium argutum* Nutt.[a] | 0 (*n* = 3) | 0 (*n* = 1) | 0 (*n* = 1) | 0 (*n* = 18) | |
| *Hieracium bolanderi* Gray[a] | 0 (*n* = 5) | | 66.7 (*n* = 6) | 0 (*n* = 15) | 33.3 (*n* = 3) |
| *Hieracium carneum* Greene[a] | 0.8 (*n* = 18) | 0 (*n* = 15) | 47.1 (*n* = 17) | 0 (*n* = 15) | 0 (*n* = 9) |
| *Hieracium fendleri* Schultz-Bip.[a] | 0 (*n* = 12) | 0 (*n* = 1) | 14.3 (*n* = 7) | 0 (*n* = 10) | |
| *Hieracium gracile* Hook.[a] | 0 (*n* = 1) | | 25.0 (*n* = 12) | 0 (*n* = 7) | 0 (*n* = 3) |
| *Hieracium greenei* Porter et Britt.[a] | 0 (*n* = 7) | | 10.0 (*n* = 10) | 0 (*n* = 14) | |
| *Hieracium gronovii* L.[a] | 3.9 (*n* = 11) | 0 (*n* = 4) | 0 (*n* = 17) | 0 (*n* = 17) | 0 (*n* = 10) |
| *Hieracium horridum* Fries[a] | 0 (*n* = 5) | 3.6 (*n* = 4) | | 0 (*n* = 3) | |
| *Hieracium longiberbe* T. J. Howell[a] | 0 (*n* = 2) | | 16.7 (*n* = 6) | 0 (*n* = 8) | |
| *Hieracium longipilum* Torr.[a] | 6.1 (*n* = 7) | 0 (*n* = 5) | 0 (*n* = 3) | 0 (*n* = 15) | 0 (*n* = 2) |
| *Hieracium parryi* Zahn[a] | 0 (*n* = 6) | 0 (*n* = 2) | 27.3 (*n* = 11) | 0 (*n* = 11) | |
| *Hieracium scabrum* Michx.[a] | 6.1 (*n* = 7) | 0 (*n* = 9) | 37.5 (*n*=16) | 0 (*n* = 15) | 0 (*n* = 2) |
| *Hieracium scouleri* Hook.[a] | 0 (*n* = 14) | 0 (*n* = 8) | 30.8 (*n* = 13) | 0 (*n* = 11) | 80.0 (*n* = 5) |
| *Hieracium venosum* L.[a] | 3.6 (*n* = 4) | | 0 (*n* = 3) | 0 (*n* = 5) | |

Values in brackets indicate the number of replicates

[a] Hawkweed species indigenous to North America.

**Table 2.** Multiple-choice host-range tests

| | *Cheilosia urbana* | | *Macrolabis pilosellae* | | *Aulacidea pilosellae* |
|---|---|---|---|---|---|
| | *Hieracium caespitosum* present | *Hieracium caespitosum* absent | *Hieracium caespitosum* present | *Hieracium caespitosum* absent | |
| Subgenus *Pilosella* | | | | | |
| *Hieracium aurantiacum* | + | | | | + |
| *Hieracium caespitosum* | + | | + | | + |
| Subgenus *Hieracium* | | | | | |
| *Hieracium canadense*[a] | + | + | | | − |
| *Hieracium umbellatum*[a] | − | + | | | − |
| Subgenus *Chionoracium* | | | | | |
| *Hieracium albiflorum*[a] | + | + | − | | − |
| *Hieracium argutum*[a] | | | − | − | |
| *Hieracium bolanderi*[a] | − | | | | |
| *Hieracium carneum*[a] | − | + | + | + | − |
| *Hieracium fendleri*[a] | | | | | |
| *Hieracium gracile*[a] | − | − | − | | − |
| *Hieracium gronovii*[a] | + | − | + | + | − |
| *Hieracium longipilum*[a] | | | | + | − |
| *Hieracium scabrum*[a] | − | − | − | − | − |
| *Hieracium scouleri*[a] | + | + | − | − | − |
| *Hieracium venosum*[a] | + | | − | | |

*C. urbana* and *A. pilosellae* were tested in the field, whereas the gall midge *M. pilosellae* was tested in 2 · 2 · 1.6 m field cages. *H. caespitosum* present: test plants and *H. caespitosum* plants were exposed simultaneously, *H. caespitosum* absent: test plants were exposed in the absence of *H. caespitosum*.

[a] Hawkweed species indigenous to North America; +, attack; −, no attack

North American *Hieracium* species, including *Hieracium carneum* and *Hieracium gronovii* (Table 1), which were also attacked in field cage tests in the presence of the target weed *H. caespitosum*. Adult midges were reared from eight native North American hawkweed species. In addition, in open-field tests carried out in 2004, the indigenous North American hawkweed species *H. carneum* and *H. scouleri* were attacked in the presence of the target weed *H. caespitosum* (Grosskopf *et al.*, 2004b). However, alien invasive hawkweeds are of increasing concern in North America (Wilson and Callihan, 1999). Recently, the European species, *Hieracium glomeratum* Froel., was recorded to also become invasive in North America (Wilson *et al.*, 2006). If *M. pilosellae* proves to be a successful biological control agent of North American target *Hieracium* species in New Zealand, a reconsideration of the risk–benefit assessment for this agent might be worthwhile.

In no-choice tests, *C. urbana* and *C. psilophthalma* develop on seven and at least two native hawkweed species, respectively. However, under open-field conditions, attack rates of *C. urbana* are much lower on the native North American *Hieracium* spp. than on the target weeds, i.e. in open-field tests, 5.6 larvae were recorded on average on *H. caespitosum* in comparison to 0.1 on *H. scouleri* and 0.3 on *H. gronovii* and *H. venosum*, respectively (Grosskopf *et al.*, 2006). Further experiments are planned to explore the level of *C. urbana* attack in the field. The low number of native *Hieracium* spp. attacked by *C. psilophthalma* is probably due to the low number of valid replicates and due to the fact that not all species have been tested. No immature stages of *C. psilophthalma* were retrieved from plants exposed in open-field tests. Due to repeated failure of open-field tests with *C. psilophthalma*, host-range investigations with this potential agent will require more time than needed for *C. urbana*.

# Conclusions

The selection of potential biological control agents for use in North America against alien invasive hawkweeds has proven difficult. We contend with a complex of target weeds and must consider potential non-target impacts on numerous native species. The most host specific of the agents tested, *A. subterminalis*, only attacks a portion of the target species, whereas less specific agents, such as *M. pilosellae*, attack most or all of the target weeds but will also infest several native species. Therefore, for a majority of our agents, we may have to balance possible non-target impacts with benefits obtained by effectively controlling the invasive species. Additional field surveys in Russia, Romania and Ukraine for stolon-attacking and gall-inducing insects are planned in the near future.

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# *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae) control in South Africa: a 10-year review

**M.P. Hill,[1] A.J. McConnachie[2] and M.J. Byrne[3]**

*Summary*

*Azolla filiculoides* Lamarck (red water fern) is a floating aquatic fern that was introduced to South Africa in 1948 and, by 1990, had infested a large number of water bodies and impacted water utilization and aquatic biodiversity. The frond-feeding weevil, *Stenoplemus rufinasus* Gyllenhal, was released against this weed in 1997. Pre-release studies showed this agent to be host-specific, damaging, capable of a high rate of population increase and had a wide thermal tolerance. The weevil was released at 112 sites throughout South Africa and rapidly dispersed to all sites of *A. filiculoides*. Quantitative post-release evaluations revealed that the weevil caused a dramatic reduction in the populations of the weed, with local extinctions occurring at the majority of the sites within the space of a year. In the last 10 years, the weed has reoccurred at a number of sites. These re-infestations did not reach the levels recorded before 1997 and were brought under control by the weevil. The weevil has shown the predicted wide thermal tolerance in the field and an ability to disperse unaided, up to 300 km. Despite local extinctions of the host plant, the weevil has been able to persist by moving between infestations of the weed. *A. filiculoides* no longer poses a threat to aquatic ecosystems in South Africa and is considered to be under complete control.

***Keywords***: *Stenoplemus rufinasus*, post-release evaluation.

## Introduction

The frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal, was imported from Florida, USA, in 1995 and was released as a biological control agent for the floating aquatic fern, *Azolla filiculoides* Lamarck (red water fern), in South Africa in 1997. Pre-release studies showed that the weevil was host-specific (Hill, 1998) and was subsequently shown to be very damaging (McConnachie *et al.*, 2004), with a broad thermal tolerance (McConnachie, 2004), and should therefore contribute to the control of the weed throughout its range in South Africa. Some 10 years after the initial releases of the weevil, we test these pre-release predictions. The review presented in this paper is based on country-wide field surveys by the authors during 1999, two in 2000, 2001 and 2006, *ad hoc* opportunistic surveys while on field trips for other aquatic weeds or from material collected by other biological control researchers, conservation bodies or land owners. In all cases, identity of the weed and the weevil were confirmed by one of the authors.

## Host specificity

*S. rufinasus* was collected on *Azolla caroliniana* Willdenow in Florida, USA, and imported into quarantine in South Africa in late 1995. As this weevil was targeted for release on *A. filiculoides* in South Africa, it was initially considered a new association (Hokkanen and Pimentel, 1984) on *A. filiculoides*, and this was used as an initial explanation for the weevil's dramatic impact on the weed. However, a recent revision of species of the American *Azolla* species based on leaf trichomes and glochidia has synonymized *A. filiculoides* and *A. caroliniana* (Evrard and van Hove, 2004). Accordingly, *A. caroliniana* now refers to *A. caroliniana sensu* Willednow (=*A. filiculoides*) and *A. caroliniana sensu*

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Mettenius (=*A. cristata*). Thus, the weevil is no loner considered to be a new association on *A. filiculoides*.

The laboratory host range of *S. rufinasus* was determined through adult no-choice oviposition and larval starvation trials on 31 plant species in 19 families (Hill, 1998). These trials showed that, while *A. filiculoides* was significantly the preferred host, some development also occurred on *Azolla pinnata* ssp. *africana* (Desv.) R.K.M. Saunders and K. Fowler, *Azolla nilotica* De Caisne Ex. Mett. and *A. pinnata* ssp. *asiatica* R.K.M. Saunders and K. Fowler. The first two species are considered to be indigenous to southern Africa, while the last species is introduced. Hill (1998) concluded that performance on these species was so poor in comparison to that on the target species that they would be unlikely to support field populations, and thus, the weevils should be cleared for release.

In the last 10 years, *ad hoc* surveys have been carried out on all three non-target species. Weevils have been recovered from *Azolla pinnata* ssp. *africana* at two sites in the Kruger National Park but in very low numbers in comparison to those found on *A. filiculoides.* The resultant impact on this non-target species is still to be quantified.

## Distribution

Temperature is one of the major factors influencing insect development (Stewart *et al.*, 1996) and has been implicated as a major contributing factor in the success or failure of biological control programmes. McClay and Hughes (1995), Stewart *et al.* (1996) and Good *et al.* (1997) have shown that failure of establishment of several biological control agents could be directly attributed to climate incompatibility of the agent to its area of introduction. *A. filiculoides* has a temperate distribution in South Africa and was especially problematic in the high-lying areas of the country where air temperature ranges between 11°C and 32°C in summer, and –9°C and 12°C in winter (Schulze, 1997). Therefore, McConnachie (2004) investigated the thermal physiology of the *S. rufinasus* to predict its potential distribution in South Africa. In the laboratory, the weevil revealed an unusually wide thermal tolerance with its lethal limits ranging from –12°C at the lowest level and 36.5°C at the upper limit: a lower developmental threshold of 9.18°C, above which it requires only 256.4 days to complete development (McConnachie, 2004). This suggested that there would be very few localities in South Africa, where the agent would fail to establish.

This prediction was upheld in that the weevil established at 91 of the original 112 (the remaining 21 sites either washed away or were not revisited) release sites, of which 53 sites were located in the cooler, high-lying above 1200 m), central regions of the country characterized by frosts in winter where the winter temperatures drop below –5°C on at least 20 nights of the year (Schulze, 1997). *S. rufinasus* has even managed to establish in the UK (Gassman *et al.*, 2006), suggesting that the agent is limited by the presence of its host plant and not by climate.

## Dispersal

McConnachie (2004) undertook a series of semi-field experiments on the dispersal ability of *S. rufinasus* and predicted that the weevil would be a moderate disperser, capable of short-distance flights among patches of *A. filiculoides*. Inference from field evidence, however, revealed that the weevil is capable of three different dispersal patterns: within site, short dispersal between close sites and long-distance dispersal. It appears as though often a single female will find a mat of the weed, oviposit and the resultant population then move out in a concentric wave destroying the mat. Once food quality declines, the adults disperse to other red water fern infestations in the vicinity (up to 20 km away) and the original mat rapidly rots and sinks. This results in mass mortality of the immature weevil stages. This behaviour has also been recorded from Florida in the United States (T. Center, personal communication). Short-distance dispersal flights of up to 20 km allow *S. rufinasus* to find most of the mats in a limited area; however, dispersal distances of up to 350 km have now been recorded (McConnachie *et al.,* 2004).

The weevil was originally released at 112 sites throughout South Africa (McConnachie *et al.,* 2004), and it has now been recorded from an additional 42 sites. The weed reoccurred at 22 of the original 112 release sites up to 2 years after the initial clearing. The weevil has since been able to relocate and clear the weed at all of these sites. Average clearance time was within 10 months. *S. rufinasus* is now widely established throughout South Africa, and there is no need to artificially distribute the agent. We underestimated the dispersal capabilities of this agent in our original predictions.

## Impact on the weed

The ultimate aim of biological control is to reduce the weed population to below an economic or ecological threshold (De Bach, 1964), but this is often not achieved using a single agent, and additional agents or intervention from other control methods are required (Stiling and Cornelissen, 2005). *S. rufinasus* was first released as 300 adults on a 1-ha pond of red water fern in Pretoria in December 1997. In January 1998, the weevils were still present, and by February, the red water fern mat had sunk and more than 30,000 adult weevils were reared from 2 m$^2$ of rotting red water fern. This dramatic population explosion occurred at all of the subsequent release sites of the agent. We were able to monitor 91 of the 112 original release sites around South Africa, all of which were completely controlled, with an average time to control being 6.9 months (McConnachie *et al.,* 2004). Where the weed has re-infested

sites from germination of spores, the weevil has relocated and controlled these infestations within a year. The weevil has not failed to control a single site where it has been released and monitored. Hill (1998) expected that "*S. rufinasus* had the potential to contribute to the control of the weed." Indeed, an additional agent, the flea beetle, *Pseudolampsis guttata* (LeConte), was imported from Florida, USA, into quarantine in South Africa and screened for release but rejected due to a lack of host specificity (Hill and Oberholzer, 2002). We clearly underestimated the impact of this weevil, and this is a good example of where a thorough pre- and post-release evaluation should be undertaken on an agent before additional agents are considered.

A full cost/benefit analysis was performed on the *A. filiculoides* biological control programme (McConnachie *et al.*, 2003), which showed that the economic returns would increase from 2.5:1 in 2000 to 13:1 in 2005, and are predicted to increase to 15:1 by 2010. Based on the field evidence, there is no reason to suggest that this prediction is an underestimate.

## Conclusion

*A. filiculoides* is considered to be under complete biological control in South Africa in that it no longer poses a threat to the biodiversity and utilization of waterways in that country. The factors that have contributed to the success of this programme include an agent that has an exceptionally high rate of increase (Hill, 1998), is a voracious feeder on the weed both as larvae and adults (McConnachie *et al.*, 2004), is a good disperser, is thermally tolerant to the South African climate (McConnachie, 2004) and does not appear to be subject to significant predation or any parasitism.

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# Species pairs for the biological control of weeds: advantageous or unnecessary?

**C.A.R. Jackson and J.H. Myers**

*Summary*

Two or more species of insects in the same genera have been introduced in some weed biological control programs, either by accident or on purpose, to increase the climatic range distribution of the agents. If the species are ecological equivalents, the introduction of both species will be unnecessary and may be detrimental if competition reduces their overall impacts. If however the species vary in the type of attack or their distributions, the introduction of congeners can be advantageous. In this paper, we review the following cases of species pair releases in the biological control of weeds in North America: the beetles *Chrysolina quadrigemina* (Suffrian) and *Chrysolina hyperici* (Forster) for St. Johnswort (*Hypericum perforatum* L.); the gallflies *Urophora affinis* Frfld. and *Urophora quadrifasciata* (Meig.) for knapweed (*Centaurea*) species; the weevils *Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner for water hyacinth [*Eichhornia crassipes* (Mart.) Solms] and the beetles *Galerucella pusilla* Duftschmidt and *Galerucella calmariensis* L. for purple loosestrife (*Lythrum salicaria* L.). In the cases of *Chrysolina* and *Galerucella*, the species pairs appear to be complementary for control of the target weeds, with each species providing good control in slightly different habitats and coexisting at some sites. With the *Urophora* gallflies, one species is more effective than the other, but overall, seed reduction is greater when both agents are present in combination (although this seed reduction is insufficient for a reduction of the target weed). In the case of the *Neochetina* beetles, a difference in the ability of the two species to kill plants was apparent, but whether they differed in habitat preferences was less clear. Given the increasing focus on the possible non-target effects of weed biological control introductions, we recommend that greater care be taken to avoid mixed species introductions and that judicious use be made of controlled field experimentation to determine impacts of the species. Molecular studies of species before introduction could help prevent the accidental introduction of multiple species.

***Keywords***: congeneric, risk, non-target effects, competition, climate, single agent, multiple agents.

## Introduction

The issue of single vs multiple agent releases in the biological control of weeds has been a subject of much debate. Some have asserted that choosing the most effective agents will reduce the potential for undesirable non-target effects (Louda *et al.*, 2003). Denoth *et al.* (2002) reviewed cases of successful biological control of weeds and found that a majority of successes are attributed to a single agent. They suggest that additional agents should be introduced only if reductions in plant density are not being achieved by the initially introduced agent(s). Where two or more agents are released simultaneously, they should be released in geographically distinct areas where the differential success of the agents can be assessed.

The success of biological control agents in reducing target plant density depends on factors such as climate, target weed phenology, nutrient conditions, dispersal ability, fecundity, and type and level of damage to key life stages of the plant. In some cases, two species in the same genus have been introduced even though they are very similar in their interactions with their host plants. This has sometimes been by accident, such as the case of the two species of *Urophora* and the mixture of the two species of *Galerucella* and sometimes on purpose from different habitats. When the species are difficult to distinguish morphologically, consider-

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able confusion can occur over the impacts, distributions and coexistence of each. This is currently the situation with *Larinus minutus* Gyllenhall. and *Larinus obtusus* Gyllenhall. introduced on knapweeds in North America (Harris, 2005).

The competitive exclusion principle predicts that two very similar sympatric species cannot occupy the same niche, and therefore, for coexistence, even very similar species must have some differences in niche requirements (Hardin, 1960). It is of interest therefore to know if differences between congeneric biological control agents contribute to their successful control of target weeds. For example, if congeneric species act in a cooperative and complementary fashion to reduce plant densities, their introductions would be warranted. The greatest advantage of congeneric biological control agents occurs if differential climatic tolerances result in effective control of the host plant across different biomes.

In this paper, we address two questions in regard to congeneric releases: (1) Is there evidence that both species are necessary for successful biological control? (2) Do congeneric species exploit different climatic regions and thus broaden the geographic effectiveness of biological control?

## Methods and materials

We restricted our analysis to agents released to control weeds in North America, based on studies reported by Coombs *et al.* (2004) and Mason and Huber (2002). Of the 25 weed control programmes reported in Coombs *et al.* (2004), 19 (76%) involved the release of more than one species of biological control agent insect, and 10 (40%) involved the release of congeneric species of agents.

For each case of congeneric biological control agent releases, we scoured the literature, searching Web of Science using agent species names as the search terms, paying particular attention to papers where the two species had been compared. We also searched for papers in the literature cited sections of each paper to find additional information.

We excluded from further analysis species pairs or combinations for which clear differences existed between the species in terms of target weeds. We also excluded species that had not yet been established or for which insufficient post-release information was available for appropriate comparisons. In addition, we excluded the *Aphthona* flea beetle species released for control of leafy spurge, *Euphorbia esula* L. Five species, *Aphthona czwalinae* (Weise), *Aphthona lacertosa* Rosenhauer, *Aphthona cyparissiae* (Koch), *Aphthona flava* Guillebeau and *Aphthona nigriscutis* Foudras, have been released in the United States and Canada, and a sixth species, *Aphthona abdominalis* Duftschmidt, has been released in the United States but failed to establish (Hansen *et al.*, 2004). The five species were introduced because of differences in habitat preferences, but control of *E. esula* in shrubby riparian areas remains a challenge (Bourchier *et al.*, 2002). Due to the large number of different species released, their recent releases and the lack of comparative data among all combinations, we did not consider this group of congeneric species. We also did not consider agents currently under consideration for release. The four species pairs discussed in detail below represent a range of habitat (grassland, riparian and aquatic) and target weed types and have (1) established, (2) reached substantial populations, (3) been studied in their non-native range and (4) been compared in the literature.

## Results

### St. Johnswort, *Hypericum perforatum* L.: *Chrysolina hyperici* (Forster) and *Chrysolina quadrigemina* (Suffrian)

The defoliating beetles, *C. hyperici* and *C. quadrigemina*, comprise two of the five species of biological control insects established in North America for control of the rangeland weed St. Johnswort (*H. perforatum*). These two beetles represent some of the earliest attempts at biological control, having been introduced in 1945 and 1946, respectively, in the United States (Piper, 2004) and in 1951 in Canada (Harris, 1962).

*C. hyperici* originates from northern and central Europe and western Asia. Eggs are laid in the fall (or in the spring in the colder continental interior; Piper, 2004). Larvae hatch and feed on leaf buds and leaves and pupate in the soil. Adults emerge in the spring, feed and then enter the soil for summer diapause before emerging in the fall to mate and lay eggs.

The native range of *C. quadrigemina* extends further south from Denmark to North Africa, and this species prefers warmer, drier areas. Both *Chrysolina* species are univoltine and have similar life cycles but slightly different phenologies.

Field and laboratory studies in New Zealand show that, for both *Chrysolina* species, the termination of summer diapause is triggered by shortening day length. However, *C. quadrigemina* terminates summer diapause approximately 3 to 4 weeks earlier, at a day length of approximately 13.5 h compared to 12.5 h for *C. hyperici*. *C. quadrigemina* females reach sexual maturity more quickly and therefore oviposit earlier. In areas with mild winters *C. quadrigemina* is considered to be the superior agent since the larvae feed on plants for a longer time than *C. hyperici* larvae (Schops *et al.*, 1996).

Early studies in British Columbia by Harris (1962) suggested that *C. hyperici* could be the superior agent in areas with early frosts. Since oviposition occurs on

average a month later than that of *C. quadrigemina*, many of the eggs do not hatch until the following spring. Thus, a greater portion of the next generation overwinters in the more resistant egg stage, thus escaping winter larval mortality.

In a subsequent assessment, Harris *et al.* (1969) found *C. quadrigemina* to dominate in dry subhumid sites, particularly those with Ponderosa pine (*Pinus ponderosa* Douglas ex Lawson et. C. Lawson), while *C. hyperici* was most effective in moist subhumid sites where Douglas fir [*Pseudotsuga menziesii* (Mirbel) Franco] was present. Both species of beetle were successful in reducing *H. perforatum* populations.

Peschken (1972) compared *C. quadrigemina* from British Columbia (BC) with *C. quadrigemina* from California (the original source of the BC introductions two decades previously) to determine if post-colonization adaptation had occurred. The BC beetles laid a larger number of eggs per female, and this could increase the number of beetles surviving the harsher winters to the next generation. Furthermore, the BC beetles demonstrated a greater tendency to seek shelter under cold temperature conditions. Although *C. hyperici* appeared initially to be the more effective agent in northern latitudes, its intolerance of dry conditions may limit its overall effectiveness.

Campbell and McCaffrey (1991) similarly concluded that, in Northern Idaho, *C. hyperici* beetles attack plants in more mesic forested areas, while *C. quadrigemina* dominates at grassland sites where the target weed *H. perforatum* most commonly occurs.

Overall, it is clear that, worldwide, *C. quadrigemina* is responsible for the majority of the reduction in *H. perforatum* populations; however, in areas with very cold winters or more mesic sites at higher elevation, *C. hyperici* offers good complementary control (Schops *et al.* 1996; Jensen *et al.* 2002).

Such prior knowledge of differences in phenology and cold hardiness could inform future agent introduction and redistribution efforts in other biological control programmes.

## Diffuse knapweed, *Centaurea diffusa* Lamark, spotted knapweed, *Centaurea stoebe* ssp. micranthos (S.G. Gmelin ex Gugler) Hayek and meadow knapweed, *Centaurea pratensis* Thuill.: *Urophora affinis* Frfld. and *Urophora quadrifasciata* (Meig.)

The gallfly, *U. affinis*, was released in Canada in 1970 and in the United States in 1973 for the control of diffuse and spotted knapweed in the genus *Centaurea* (Harris, 1980a; Story *et al.*, 1987). *U. affinis* oviposits in immature flower heads, inducing the formation of a woody gall in the receptacle, and reduces knapweed seed production (Harris, 1980b; Shorthouse, 1989). Another gall fly, *U. quadrifasciata*, was accidentally released in 1973. *U. quadrifasciata* oviposits in slightly larger, more mature flower heads, induces the plant to form a thin, papery gall in the ovary and also reduces seed production (Harris, 1980b).

*U. affinis* is primarily univoltine, whereas *U. quadrifasciata* is partially bivoltine. In Western Montana, the peaks of first-generation emergence of the two species occur approximately 1 week apart: 25 June for *U. affinis* and 2 July for *U. quadrifasciata* (Story *et al.*, 1992). At the time of fly emergence, the majority of knapweed flower heads are at the most suitable stage for *U. affinis.* Attack by *U. affinis* stunts the growth of the remaining heads so that many do not reach the size acceptable to *U. quadrifasciata* (Berube, 1980), and thus they must disperse to find sites with capitula suitable for oviposition for the second generation (Harris and Myers, 1984; Story *et al.*, 1992; Mays and Kok, 2003). This could account for the broader distribution of *U. quadrifasciata* in Canada and the United States (Story and Coombs, 2004a,b) despite *U. affinis* being less active fliers (Roitberg, 1988). In a survey in Montana, *U. quadrifasciata* occurred at almost all sites examined as compared to *U. affinis* that was found at approximately half of the sites (Story *et al.*, 1987).

Surveys of original release sites in British Columbia have revealed that *U. affinis* is most often the dominant species (Harris, 1980a; Myers unpublished data), in agreement with predictions made by Story *et al.* (1992). However, because of geographic and annual variation in flower head sizes at emergence times, it is unlikely that *U. affinis* will eventually displace *U. quadrifasciata* (Berube, 1980). In addition, the supercooling capacity of *U. affinis* is superior to that of *U. quadrifasciata* (Story *et al.*, 1993), and Nowierski *et al.* (2000) conclude that *U. affinis* is more tolerant to cold winter temperatures. This could also help to explain the predominance of *U. affinis,* particularly at the more northerly release sites.

The exception to *U. affinis* dominance is in southwest Virginia, where surveys have shown that *U. quadrifasciata*, which was not introduced but is thought to have dispersed from releases in Maryland, now outnumbers *U. affinis*. The longer growing season in this area may favour *U. quadrifasciata,* although it does not appear to have displaced previously established *U. affinis* populations (Mays and Kok, 2003).

*U. affinis,* although smaller than *U. quadrifasciata* (Roitberg, 1988), reduces seed production by 2.4 seeds/head in comparison to 1.9 seeds/head for *U. quadrifasciata* and thus could be considered to be a more effective agent (Harris, 1980a). Myers and Harris (1980) found that overall seed reduction was slightly greater when both agents were present in combination. As *Centaurea* is not seed-limited, however, these gallflies have not been successful in reducing overall weed density (Myers and Risley, 2000).

## Water hyacinth, *Eichhornia crassipes* (Mart.) Solms: *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache

The weevil *N. eichhorniae* was released in the southern USA in 1972. The adults and larvae feed on water hyacinth (*E. crassipes*) leaves, often damaging meristematic tissues, and leaving distinct scars which limit plant growth. *N. bruchi* feeds in a similar manner. Both species are multivoltine (DeLoach and Cordo, 1976; Center, 2004).

Both *Neochetina* species are capable of reversibly generating and degenerating flight muscles to correspond with dispersal and reproductive phases. *N. bruchi* appears to be more sensitive to plant quality and require high nitrogen content to sustain their high fecundity (Spencer and Ksander, 2004). On stressed plants, Center and Dray (1992) found more *N. bruchi* with developed flight muscles than *N. eichhorniae.* Differences in preferences for oviposition sites may decrease interspecific competition and lead to complementary effects, as *N. eichhorniae* prefers to oviposit on younger more central leaves, while *N. bruchi* prefers to oviposit on older, more outer leaves (DeLoach and Cordo, 1976).

*N. bruchi* appears to be the superior agent, mainly because it has a faster population growth rate, the females lay more eggs and the larvae develop faster. Thus, these beetles can kill plants faster than *N. eichhorniae* (DeLoach *et al.*, 1976). The two species occur together throughout their native range, although *N. eichhorniae* predominates at warmer latitudes in northern Argentina, Paraguay and Brazil. Also, *N. eichhorniae* is more tolerant to extremely high temperatures for oviposition, adult feeding and adult and egg survival. *N. bruchi* adults, in contrast, survive better at lower temperatures (DeLoach *et al.*, 1976). In Florida, *N. eichhorniae* is the more widespread agent (Center *et al.*, 1992; Center *et al.*, 1999), and it is this agent that is most often credited with the control of *Eichhorniae crassipes* in North America (Goyer and Stark, 1984; Center and Durden, 1986; Center, 1987).

It is possible that *N. eichhorniae* may be more suitable at warmer latitudes, while *N. bruchi* may be more effective at cooler temperatures and under higher nutrient levels (Heard and Winterton, 2000).

## Purple loosestrife, *Lythrum salicaria* L.: *Galerucella pusilla* Duftschmidt and *G. calmariensis* L.

The defoliating beetles, *G. pusilla* and *Galerucella calmariensis* were introduced in North America as mixed releases in 1992 (Hight *et al.*, 1995). Adults and larvae feed on the buds and leaves of purple loosestrife (*L. salicaria*) killing plants or reducing their vigour in subsequent years. The two species of beetles are very similar in life history strategies and appearance, and they can only be distinguished by dissection of the males. In a study of the beetles in their native range, Blossey (1995) concluded that these very similar species, which make identical use of their shared food resource, are able to coexist. He proposed that coexistence could be due to differences in the competitive abilities of individuals, as proposed by Begon and Wall (1987): Individual variation, rather than niche differentiation, promotes the coexistence of competing species.

In a survey of Central New York completed in 2004, 10 years after the initial introduction of the beetles, Grevstad (2006) found that *G. pusilla* was generally more abundant than *G. calmariensis* but that the abundance of *G. calmariensis* was increasing. Grevstad concluded, however, that the two species did not occupy identical niches and that coexistence could be due to the greater dispersal abilities of *G. calmariensis*, which allows the beetle to coexist with an almost identical competitor, *G. pusilla*.

McAvoy and Kok (2004) found that the phenologies of both species are almost identical and are well synchronized with the host plant. One species completes egg development more slowly, while the other species has faster larval development. In contrast to *Neochetina* beetles, neither beetle species exploits the lower quality food source of older leaves.

McAvoy and Kok (2004) concluded that, at higher, colder latitudes in North America, the overall better cold tolerance of *G. calmariensis* makes it a superior competitor. The situation is complex, however, as *G. calmariensis* larvae feed more at lower temperatures, but *G. pusilla* larvae may survive better when food is limiting, as they require less food for development (McAvoy and Kok, 2007).

In western Oregon and Minnesota, *G. pusilla* is the dominant species (Schooler, 1998; L. Skinner, personal communication), although *G. calamariensis* is more common at northern locations in Minnesota (L. Skinner, personal communication). In Michigan, where mixtures of the two species were originally introduced, based on morphological identification, only *C. calmariensis* currently occur (Landis *et al.*, 2003; D. Landis, personal commnunication). In Canada, beetles initially came from the mixed species stock in the United States. In 2005, it was found that in, Ontario, *G. calmariensis* dominated sites of mixed releases that had been made in the mid-1990s (J. Corrigan, personal communication). This is similar to observations in Michigan and Minnesota but conflicts with the continued dominance of *C. pusilla* in New York. In western Canada, species identifications were not confirmed but are thought to be *G. calmariensis* (Lindgren *et al.*, 2002; Denoth and Myers, 2005).

The difficulty of distinguishing the two *Galerucella* species complicates analysis, but *G. calmariensis* seems to be the superior agent in terms of dispersal and persistence particularly in the North. Successful biological control apparently occurs with either species alone or together.

## Discussion

Biological control is highly context-dependent. Agent persistence and plant damage depend on target plant nutritional status and phenology. Furthermore, insect agents are highly sensitive to local climatic conditions of temperature, precipitation and humidity. With increasingly variable trends in climate due to global change, having more than one agent could serve as an insurance policy against fluctuations in survival due to environmental conditions.

The two *Chrysolina* beetle species seem to offer complementary control of St. Johnswort on a continent-wide scale. Overall, *C. quadrigemina* is probably responsible for most of the control of the weed, with *C. hyperici* offering complimentary control in more mesic, higher elevation and forested sites. This pattern is similar to the latitudinal gradient in *Galerucella* spp., with control of purple loosestrife by *G. pusilla* being dominant at more southerly locations, and *G. calmariensis* dominant at more northerly locations. In the case of the *Urophora* gallflies, although *U. affinis* is superior in terms of seed reduction, overall seed reduction is slightly greater with the two species, but the seed reduction is insufficient for successful biological control.

Caution, however, is advised. In some cases, competition from an inferior agent can result in reaching suboptimal levels of control (Crowe and Bourchier, 2006). Furthermore, under certain environmental conditions, it may be best to introduce only one or other of the agents. For example, under high nutrient conditions, the release of *N. bruchi* could result in greater control than the release of *N. bruchi* in combination with *N. eichhorniae*.

Along with the risk of achieving sub-optimal control due to competition from similar species, with each new species introduction comes the risk of non-target effects on ecosystems, a subject which has received much attention of late (Cory and Myers, 2000; Strong and Pemberton, 2000; Louda *et al.*, 2003). We conclude that the best strategy is careful field and laboratory pre-release experimentation in the native habitat, followed by the evaluation of replicated releases of individual species into geographically distinct areas. Finally, we recommend that, in addition to maintaining voucher specimens of insect releases, molecular tools for species identification be developed so that mixed stocks of species and strains can be identified.

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# Field studies of the biology of the moth *Bradyrrhoa gilveolella* (Treitschke) (Lepidoptera: Pyralidae) as a potential biocontrol agent for *Chondrilla juncea*

**J. Kashefi,[1] G.P. Markin[2] and J.L. Littlefield[3]**

*Summary*

The root-attacking moth, *Bradyrrhoa gilveolella* (Lepidoptera: Pyralidae), has been released as a biological control agent for *Chondrilla juncea* L. (Asteraceae) in Argentina and Australia; however, both efforts failed. As part of our effort to establish this insect in North America, we conducted a field study of its biology in northern Greece with the goal of making an informed release according to the most suitable environmental conditions for the larvae and to synchronize the phenologies of this insect with its host. Our study population was at 950 m in the mountains of northern Greece, an area climatically matching the interior mountains of the state of Idaho (USA), our intended target for colonization. Besides obtaining a much more complete picture of its basic field biology, the most significant finding of this study was that despite living in an area with a long, cold, snow-covered and extensive winter, and very hot summers, this insect has a single generation a year but no distinct over-wintering stage or highly synchronized period of emergence of adults during the summer.

***Keywords***: *Chondrilla juncea*, rush skeletonweed, *Bradyrrhoa gilveolella*, field biology.

## Introduction

*Chondrilla juncea* L. (Asteraceae) is a long-lived perennial of European origin with a deep taproot. A rosette during most of the year, in early summer it bolts to produce a tall, woody forb with slender, leafless branches, hence the common name 'skeletonweed'. Its natural range in Europe extends from Volga River in Russia to the Atlantic coast, and surrounds the Mediterranean Sea. It is a major weed in Australia, Argentina, and the northwest United States and adjacent Canadian provinces (Holm *et al*., 1997). The most recent and rapidly spreading population in North America and the one being targeted for control is in the southwest corner of the state of Idaho. In Argentina and Australia, the plant is a major problem in wheat fields, but in Idaho, it is still primarily restricted to native ecosystems being managed by the United States Forest Service particularly in drier pine forests and on open hillsides.

Biological control was initiated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia, which released and established three agents: a blister-forming Cecidomyiid midge, *Cystiphora schmidti* (Rübsaamen), a gall-forming Eriophyid mite, *Eriophyes chondrilla* (Canestrini) and the rust fungus *Puccinia Chondrilla* Bubak & Sydenham (Julien and Griffiths, 1998). The programme was initially very successful, due to the action of the rust (Burdon *et al*., 1981; Cullen, 1981). In North America, the same three agents were introduced and are presently established throughout the range of *C. juncea* (Piper *et al*., 2004). Unfortunately, in southern Idaho, their combined effect is having little or no discernible effect in reducing the spread or impact of this invasive weed.

The Australians also released a fourth agent, the root-attacking moth *Bradyrrhoa gilveolella* (Careshe and Wapshere, 1975); despite numerous attempts, however, it failed to establish (Cullen, 1981). With the apparent successful control of *C. juncea* by the rust, Australian authorities eventually discontinued work on this agent. Subsequently, the Argentinians attempted to establish *B. gilveolella* but also failed (Julien and Griffiths, 1998). In Idaho in the mid 1990s, due to the

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rapid spread of *C. juncea* and its impact on native ecosystems, a new effort at biological control was initiated and the first agent selected was *B. gilveolella,* based on its known specificity as shown in the work conducted by Australians (Careshe and Wapshere, 1975). A petition to release *B. gilveolella* was submitted for release in North America (Littlefield *et al*., 2000) and approved in 2002. Attempts are presently underway to establish this insect in Idaho. In view of its failure to establish in both Argentina and Australian, we realized that this may be a difficult insect to colonize. Accordingly, concurrent to our attempt to establish it in Idaho, we have been studying both its habitat preference (Littlefield *et al.*, 2008) and its biology under field conditions in northern Greece. We are hoping to identify clues to help synchronize the life stage of *B. gilveolella* being released with the phenology of *C. juncea*, the local climate of southern Idaho, and other factors which might affect its establishment, such as coarseness of soil.

# Methods and materials

## Targeted release sites in Idaho

Within the south-west Idaho infestations, Garden Valley, 50 km north of Boise, was selected as the target area. The introduction of *C. juncea*, originally carried out in the mid 1960s, in this valley represents the oldest and densest stand of *C. juncea* in southern Idaho.

## Collection site in Europe

To find a *B. gilveolella* population in Europe living under a similar habitat and climatic zone to Garden Valley area, we examined populations of *C. juncea* around the Black Sea and throughout the Balkans and found *B. gilveolella* at many sites. We then attempted to match habitats in Europe with our target areas in Idaho by comparing soil temperatures. At five locations within the south-western Idaho *C. juncea* population, soil temperature probes (‘Optic Stow-Away Temp Probes’, Onset Computer Corporation, Pocasset, MA, USA) were buried 2–3 cm deep (where the *B. gilveolella* spends its larval stage) at the base of mature *C. juncea* plants. Probes were similarly buried at seven sites in Bulgaria and northern Greece. Based on a comparison of habitats (Littlefield *et al*., 2008) and soil temperatures, we selected an area at Lake Prespa in north-western Greece for collections. This high (950 m) mountain valley has a long, cold winter, usually with a prolonged snowpack, and has the closest soil temperature match to Garden Valley in Idaho (Figure 1). The large population of *B. gilveolella* found in the sand beach around this lake has become the source of the populations being tested and introduced to Idaho. We have also concentrated on studying the insect’s biology in this area.

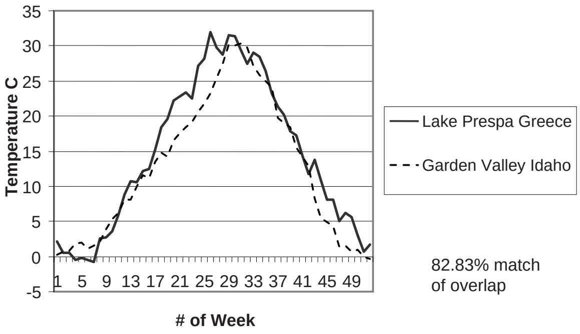


**Figure 1.** Composite for mean weekly soil temperature at 2–3 cm depth for 2002 to 2005. The profile for Garden Valley is laid over the profile for Lake Prespa.

## Seasonal life history of *B. gilveolella*

Field sampling consisted of fortnightly visits to Lake Prespa, where the sampling area was divided into three parallel collection strips, each about 800 m long and 20 m wide according to the fineness of the sand grains (coarse, top of beach; medium, middle of the beach; fine, near the water). Field researchers walked through one randomly selected transect at each collection strip, and the closest *C. juncea* plant at every 3-m interval was excavated. There were higher densities of plants near the lake, and medium densities in the other two sampling strips away from the water. At each sampling time, a total of 51 plants from all three sampling strips were collected. In the European Biological Control Laboratory (EBCL) substation in Thessaloniki, Greece, the larvae were extracted from their feeding tubes and their instar was determined. All larvae were preserved in alcohol, and 533 were found suitable for head capsule measurements to determine their development stage. The total number of larvae collected each month over the 4 years was determined and the percentage of each of the five instars calculated. Additional studies on the biology of this insect were made in quarantine facilities in Bozeman, MT, USA, while it was undergoing host specificity testing (Littlefield *et al*., 2000).

# Results

Examination of larvae in the field has given us information on their stage of development at different times of the year, their method of over-wintering and time of adult emergence (i.e. the presence of pupae or recently empty pupae cases). Figure 2 shows the relative abundance of the different instars of *Bradyrrhoa* found at Lake Prespa over the 4 years of this study. Pupae were found beginning in mid June, peaking in July, and ending in early August. Presumably, adults were present in the field at about the same time.

Adult females mate immediately after emerging but usually require 2–3 days of feeding on sugar water in

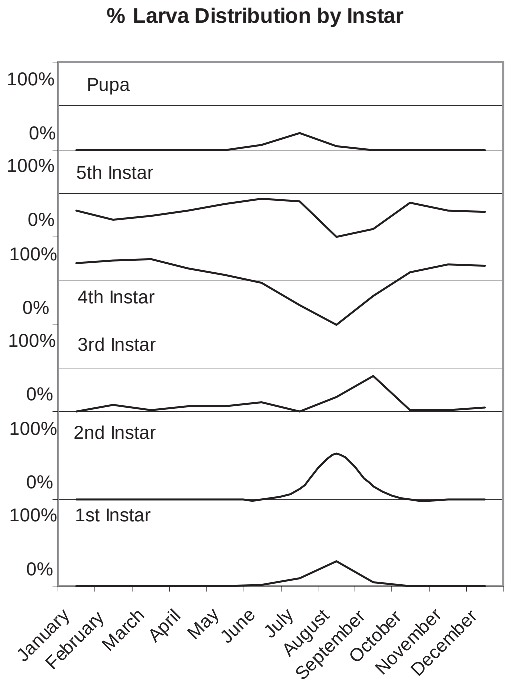


**Figure 2.** Yearly occurrence of the various life stages of 533 *Bradyrrhoa gilveolella* larvae collected in the field at Lake Prespa. Data used is based on the monthly cumulative number of larvae collected between 2003 and 2005.

the laboratory before they begin oviposition. The ratio of adult males to females is approximately equal. In the laboratory, mating takes place readily in small mesh emergence cages or plastic storage boxes containing only paper towels. Oviposition in the laboratory lasts 5–7 days with a female producing on average 100 eggs (Littlefield *et al*., 2000).

## Eggs

In mesh cages over potted plants in the greenhouse, females do not have a preferred oviposition site, and eggs were found on a wide variety of plant parts. Unfortunately, despite intensive searching we did not find eggs or empty egg shells at Lake Prespa so we cannot confirm the normal location for oviposition in the field. In the greenhouse on caged plants, eggs are firmly attached to the plant part that they are laid on, are 0.5–0.7 mm in diameter, flattened, and initially white, but as they develop, become reddish, then darken 3–5 days before hatching as the larvae becomes viable.

## Larvae

Results of 3 years of field observations showed that 620 (30.4%) of the total larvae counted were collected from the transect with plants growing in coarse sand, 48.4% in mixed sand and 21.2% fine sand.

***First Instar*:** The newly emerged larva descends from the plant by crawling down the leaf or stem to the rosette. There it works its way down the space between the crown and the soil and chews a small cavity approximately 0.5 mm in diameter into the root 2–3 cm below the soil surface. From this wound, the plant exudes latex, which penetrates into the surrounding soil forming a porous clump. The larva lives in cavities in this clump but does not form a distinct feeding chamber.

***Second Instar*:** The larva remains in the latex clump and continues to feed in small pits in the root and forms a chamber 1 mm in diameter and 3–4 mm long adjacent to the root surface.

***Third Instar*:** The larva abandons the latex clump and feeding pits, and at a slightly lower point on the root constructs the first true feeding chamber: a small 1- to 2-mm silk-lined tunnel attached to the outside of the root. Feeding is restricted to a feeding groove 1–2 mm wide and 3–5 mm long, penetrating the root cortex. The larva feeds on the edges of this groove which have a distinct yellow colour but show no sign of the latex that, otherwise, oozes from any wound in the cortex.

***Fourth Instar*:** The larva feeds by enlarging the groove excavated in the root but a major change occurs in the feeding tube. It is extended upwards to the soil surface, either along the side of the plant or as a branch from the main feeding tube. The feeding tube reaches 10 cm or more in length, and by moving within it, the larva escapes extremely low soil temperatures to survive during winter when temperatures occasionally reaches –10°C. Feeding is at a single feeding site which expands to a groove up to 10 cm and more in length and 2–3 mm wide. However, the depth remains shallow (1 mm), and shows the distinct yellow edge where the larva has cut into the cortex of the plant.

***Fifth Instar*:** The majority of the larval growth (at least 50%) occurs in this instar. There is no significant change in the size or shape of the feeding tube although the larva continues to strengthen and thicken the wall using pellets of latex. Feeding remains concentrated in the original feeding groove, which reaches its maximum length of 10–15 mm. Upon completion of feeding, the opening between the tube and feeding groove is closed with silk and the interior of the feeding tube is lined with an additional layer of silk and latex to form the pupal chamber.

## Pupa

Pupation occurs in the special silk-lined chamber in the feeding tubes, with all exuviate and frass sealed below. Upon completion of pupation, which in our greenhouse requires 7–14 days, the newly emerged adult crawls up the feeding tube or its side branch. The end of the tube consists of only a light layer of silk covered with soil particles, which the adult traverses to emerge at the soil surface.

## Feeding tube

The most distinct feature in the development of this insect is the rather unique feeding tube attached to the *C. juncea* root. Initially, this is a fine, silk-lined tunnel constructed by the third instar larva down the outside of the root of the plant. The length of this small tunnel apparently dictates the size of the subsequent feeding tube since as the larvae grow, they do not appear to extend the tube, but only enlarge its diameter and strengthen the wall with latex pellets. In loose loam or fine loam, the wall of the tube is thin, flexible and almost cloth-like, but in coarser soil and sand, grains are incorporated into the wall making it much thicker and firmer. The length of this feeding tube and the larvae in the soil are quite variable, but at Lake Prespa it averaged 7.12 cm (range 1–21 cm). The method of constructing the tunnel in which the tube is formed is unknown. It is not excavated since the tunnel initially does not reach the soil surface, so it possibly formed by the larva forcing the sand grains or soil particles apart. The tunnel is subsequently lined with the latex particles and layers of silk, and in the fourth and fifth instar, an excess of latex particles is often produced which often form a distinct clump of white pellets in the bottom of the feeding tube. The latex content of this feeding tube attracted the attention of Russian scientists who discovered that it was a high-quality natural rubber, and studied *B. gilveolella* (Kozulina and Rudakona, 1932) to determine if these feeding tubes could be harvested as a source of locally produced rubber (Dirsch, 1933; Iljin, 1930).

## Feeding

Any slight injury to the root of *C. juncea* results in a copious flow of highly viscous white latex. *B. gilveolella* is highly co-evolved with the plant and not only can compensate for this latex flow but may utilize it as food. The feeding groove the larva creates in the root's cortex is often no larger than the body of the larva and after the second instar there is no sign of an uncontrolled flow of the latex. Apparently, the larva, feeding on the outer edge of this groove, ingests the latex along with other nutrients produced by the plant and excretes the latex as small, distinct, white oval pellets, which it incorporates into the wall of its feeding tube. The limited size of the groove itself appears to indicate that the larva does not obtain its nutrients by consuming significant quantities of root tissues, but must subsist on the sap and latex flowing from this wound.

## Impact

In the field, there is no visible impact on mature plants from the feeding of several larvae except on very small plants (roots, 1–2 mm in diameter) which are severed and killed, resulting in starvation of the larva. In the field, the insect shows no preference for plant size, and there seems to be uniform attack on plants with roots as small as 2 mm up to 20 mm in diameter. Since the larvae do not consume significant amounts of root tissue, their impact appears to be primarily through stressing the plant by diverting the nutrients that would normally go into growth.

## Generations per year

In early studies in Russia, *Bradyrrhoa* was found to have two widely overlapping generations per year (Kozulina and Rudakova, 1932). Subsequently, an Australian study on the coast of Greece also reported two generations per year (Careshe and Wapshere, 1975). At Lake Prespa, we were initially confused since sampling in spring or fall, produced a very wide range of larvae (from as small as 5 mm to more 20 mm), but without clear evidence of two generations. Once we began to separate the larvae by instars and began sampling during the summer months, it became clear that we had only a single generation per year. Adult emergence begins in mid-June and continues for almost 2 months. At this time, the larger larvae (fourth and fifth instars) disappear, marking the end of the first generation. First and second instar larvae are found only from July to September. Apparently, the first larvae to hatch complete most of their development and enter diapause in early November as fifth-instar larvae, but those larvae that hatch from the last eggs laid enter diapause as fourth-instar or, a few, as late third-instar larvae. Larvae end diapause the following spring and complete their feeding and development by that summer to give rise to the next generation.

# Discussion

Soil temperature comparisons between Lake Prespa, Greece, and the Garden Valley sites in Idaho appeared to be the closest match between Idaho and any of the areas studied in the Balkans. *B. gilveolella* over-winters as a late fifth-instar larva or as a pre-pupa, along the coast of Greece, where it has two distinct generations per year (Careshe and Wapshere, 1975). At Lake Prespa, the population has no distinct over-wintering stage and hibernates as any of the last three instars. Since an active larva would possibly be susceptible to freezing temperatures, this would seem to be a disadvantage. However, its survival is probably dependent on the snow pack that covers the ground surface for the majority of the coldest part of winter. Similarly, our site in southern Idaho has a similar snow cover which should protect these larval stages.

Based on these observations, we recommend that if first instar larvae are to be released in Idaho, they should be released in July or August. However, soil surface temperatures during the day can be extremely high, so late evening releases of some type of shading may be necessary. Late-instar larvae (fourth or fifth)

should be released in September or October. In all the above-mentioned cases, the surface of the release area should be covered with loose sand to a depth of about 5 cm to allow fast and easy penetration of the larvae into the ground and to protect them from heat and dehydration. If releases of adults are planned, they should be in cages in July.

## Acknowledgements

Since Lake Prespa and the surrounding valley lay within a Greek national park, we are very grateful to the Greek Ministry of Agriculture, Office of Development and Protection of Forests and Natural Habitat and CITES office, Region of Central Macedonia, Thessaloniki, for authorizing our study and issuing us collection and export permits for *C. juncea* and *B. gilveolella*. In quarantine at Bozeman, Montana, we are very grateful to technician Annie Demeij and graduate student Heather Prody for assistance in rearing and maintaining our colony and helping us with the laboratory studies.

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# The release and establishment of the tansy ragwort flea beetle in the northern Rocky Mountains of Montana

**J.L. Littlefield[1], G.P. Markin[2], K.P. Puliafico[3] and A.E. deMeij[4]**

*Summary*

The flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae), has successfully suppressed tansy ragwort [*Senecio jacobaea* L. (Asteraceae)] in mild, mid-latitude climates of western North America. Attempts to establish this flea beetle in more continental climates, such as those found in the interior of the Pacific Northwest, have failed. With the recent incursion of tansy ragwort in northwestern Montana, a biological control program was implemented. Two populations of *L. jacobaeae* from Oregon (collected from low and high elevation sites) and cold-adapted populations from Switzerland were released between 1997 and 2006. Several release techniques using flea beetle eggs, larvae or adults were tried, and those using eggs or larval flea beetles were less successful than those using adults. Subsequent surveys indicate that the Oregon low-elevation population failed to establish and that the Oregon high-elevation and Swiss populations have established and are dispersing from their original release sites.

***Keywords***: tansy ragwort, ragwort flea beetle, *Longitarsus jacobaeae*.

## Introduction

The Eurasian weed species, tansy ragwort, *Senecio jacobaea* L. (Asteraceae), readily invades disturbed rangelands, pastures, open forests and other natural areas in areas of the western USA. Montana was considered tansy ragwort-free until several infestations were located in Lincoln and Flathead Counties after the 1994 Little Wolf Fire (USFS, 1996). By 1997, despite extensive treatments with herbicides, the plant was found to be too widely distributed and well established to be eradicated or economically controlled. Several biological control agents were initially introduced by the US Forest Service from established sites in Oregon (Markin and Birdsall, 2002); including the cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), the tansy ragwort seed fly, *Botanophila seneciella* (Meade) (Diptera: Anthomyiidae), and the tansy ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae). An additional population of the flea beetle was later introduced from Switzerland. This paper reports on the release and successful establishment of two populations of the flea beetle in Montana.

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## Methods

### Flea beetle populations

Three populations of the tansy ragwort flea beetle were introduced into Montana. Our initial attempt to establish *L. jacobaeae* used beetles from established populations in western Oregon. These flea beetles (referred to as the Oregon low-elevation population) were collected from the Willamette Valley (elevation, 75 m) or along the Oregon coast near Florence. This population was established from the Italian strain of the flea beetle collected near Rome, Italy in 1968 by K. Frick (USDA-ARS) and initially introduced into northern California in 1969 and in Oregon in 1971 (Frick, 1970; Hawkes, 1980). This population has been very effective in controlling tansy ragwort in coastal areas of

Oregon, Washington and northern California (Hawkes and Johnson, 1978; McEvoy *et al.*, 1991). The seasonal life cycle of the Italian strain has been reported by Frick and Johnson (1973) and Windig and Vrieling (1996). Key aspects of this phenology are (1) the adults emerge during the summer and undergo a summer aestivation, (2) adults re-emerge in the autumn with the onset of seasonal rains and begin to oviposite, (3) oviposition continues through the winter months, (4) eggs do not have a diapause and hatch within 10 days and (5) both eggs and early instar larvae can be observed during the winter months. This phenology, although well suited to milder, mid-latitude climates, is ill-adapted to colder, drier, continental climates. Previous introductions of *L. jacobaeae* in the interior portions of the Pacific Northwest, east of the Cascade Mountains, have not been successful (Coombs *et al.*, 1996), and we suspected that results for Montana would be similar.

A second population of the Italian strain was also introduced from Oregon. This population (referred to as the Oregon high-elevation population) was collected from Mt. Hood, Oregon (elevation, 1100 m), and differs in phenology from the Oregon low-elevation population in that adults emerge in late summer and eggs are the primary overwintering stage (Hawkes, 1980; Markin and Birdsall, 2002). Although it has adapted to colder climates found on Mt. Hood, population density of this flea beetle appear to be less than at lower elevation sites (Markin and Birdsall, 2002).

A third population of *L. jacobaeae* from Switzerland was investigated, as it had been reported to be better adapted to continental climates (Frick, 1971). The life history and biological attributes that would make this population more cold-adapted were investigated by Frick (1971), Frick and Johnson (1972) and Puliafico (2003). The phenology of the Swiss population of *L. jacobaeae* differs from that of the Italian population in that (1) adults emerge in the later part of the summer (e.g. starting in mid-July) and do not aestivate, (2) oviposition occurs after 2 weeks and the beetles overwinter as eggs in a semi-diapaused state and (3) larvae hatch the following spring and complete their development by mid-summer. Host-specificity testing of *Senecio* and *Packera* species endemic to northwestern Montana indicated no significant non-targets impacts associated with the Swiss populations of *L. jacobaeae* (Puliafico, 2003), and therefore, the beetle could be introduced safely into Montana. Flea beetles were collected in 2002 to 2004 by U. Schaffner (CABI-Europe) from St. Imier and Mettembert, Switzerland, from elevations of 820 and 640 m, respectively.

## Release methods and monitoring

Several different release techniques were used to establish *L. jacobaeae* in Montana. Adults were used for the releases of the Oregon low-elevation population. Beetles were collected from sites in Oregon in early September and were field-released within large cages (2 × 4 × 2 m) or smaller cages (1 × 1 × 1 m). *L. jacobaeae* collected from Switzerland were screened before release for possible parasites, pathogens and other flea beetle species at the Montana State University Biological Control Containment Facility. Eggs obtained from field-collected adults were used for initial releases and for maintaining a laboratory colony for subsequent releases. Initial releases of the Swiss population used eggs. Eggs were harvested and placed in groups of 25 eggs on a strip of filter paper, which was placed next to the root crown of tansy ragwort plants at field sites and held in place by moistened peat moss or soil. Eggs were placed in the field in early November and, after cold treatment, in late April or early May, June and July. In subsequent years, we also inoculated plants with newly hatched larvae, or adults were released uncaged or into cages (2 × 4 × 2 m). The Oregon high-elevation populations were released as adults in cages and also as larvae transplanted into the field in infested plants from a laboratory colony or as larvae in infested plant material, which was placed on tansy ragwort in the field.

The success of releases was determined by the collection and dissection of plants throughout the summer to look for larvae or to determine larval feeding. This was conduced during the year of release and the following year. Adults were vacuumed sampled on a yearly basis using a modified leaf blower. Sites were sampled from late July through late October or early November. Due to uneven plant densities, adult counts were expressed as adults per 100 plants.

# Results

## Oregon low elevation population

Releases were made in 1997 and 1998, with a total of 435 adults released at six sites in Flathead County. Approximately 170 to 280 adults were released per site, although numerous smaller releases of 10–15 adults were also made. Adults were recovered at low levels for 2 or 3 years after release. Flea beetle feeding was observed in early September, and adults were observed from mid-September to mid-October. By 2005, no adults were recovered at release sites. During this time, the tansy ragwort density at sites significantly declined due to cinnabar moth feeding (Markin and Littlefield, 2006). This decline may have impacted any flea beetle populations remaining at these sites. To our knowledge, no long-term establishment of this population has occurred in Montana.

## Oregon high elevation population

Before the release of the Swiss population, a second population of *L. jacobaeae* was located on the slopes of

Mt. Hood, Oregon. Releases were made starting in 1999 and continued through 2001. An estimated 410 adults and 2905 larvae were released at 20 sites in Flathead and Lincoln Counties. Releases made in 1999 were inadvertently sprayed with herbicides, and no recoveries of the flea beetle have been made from these sites. Based on observations of the flea beetle habitats on Mt. Hood, releases in 2001 occurred in more mesic habitats along the Little Wolf drainage in Lincoln County. By 2002, larvae were recovered at four sites where either adult or transplanted infested plants were placed. In 2006, we determined that the Oregon high-elevation population had increased in numbers and dispersed several hundred metres from the initial releases in the Little Wolf drainage. Flea beetles were most evident in moist micro-habitats. At one site, adults were sampled along a moisture gradient, starting at a wet seep (the site of release), then progressing out 60 m into a drier habitat. The number of adults was higher along the wet seep (92 beetles per 100 plants) but decreased rapidly (two to nine beetles per 100 plants) as one moved into drier areas. We are not certain if the presence of adults in the wet area was due to available water, greater plant density or if moisture conditions are favourable for egg laying and/or survival.

## Swiss population

A total of 27,560 eggs, 16,435 larvae and 2937 adults have been released at field sites from 2002 to 2006. Several release techniques were used in our effort to establish flea beetles. Our initial releases (2002–2004) were made using eggs obtained from our rearing colony. As large numbers of eggs could be collected, we thought this would be an efficient way to release the insect. This technique did not appear to be successful due to low establishment. In 2005, we switched tactics by releasing larvae that had just hatched and eggs that were about to hatch. Larvae or eggs were placed on tansy ragwort rosettes or at the base of developing stems. This technique proved more difficult, as timing of the larval hatch was critical and a large number of larvae had to be placed on plants within a short time span.

With improvements in our flea beetle rearing, we were also able to release adequate number of adults for the first time in 2005 and 2006. Adult releases have several advantages in that they are less time consuming, adults are less vulnerable to handling damage and they are more likely to select oviposition sites that maximize offspring survival.

In general, the technique of using eggs to inoculate plants proved unsatisfactory. Although larvae were recovered from plants with autumn and spring inoculations, no recoveries were made on those inoculated in June or July. It is speculated that eggs may have desiccated, were more susceptible to predation or the plants were unsuitable for larval establishment at these later dates. Of the 21 sites with egg inoculations, three have recoverable beetle populations after 4 years.

With the success of the cinnabar moth on the Flathead County side of the tansy ragwort infestation, most of the sites were abandoned due to very low tansy ragwort plant densities. However, there was indication that the flea beetle had established before the rapid decline of tansy ragwort. Two sites in Flathead County were retained, as these appeared to have persistent pockets of tansy ragwort, and adults of the Swiss population were released at these sites in 2005. In Lincoln County, where the cinnabar moth has not been as successful, at two sites that received larvae in 2005, adults were recovered, and of the ten adult releases made before 2006, adults were recovered at nine.

Thirty-two release locations (a location may be comprised of several individual release plots) were visited in August and September 2006. Flea beetle adults were observed at 23 locations (72% of the locations). Beetles were observed at all, except for one, of the 2005 releases. At selected sites in the Little Wolf drainage in Lincoln County, adult beetles were collected and returned to the laboratory to confirm their population origin. From egg hatch data, it appeared that both the Swiss and Oregon high-elevation populations (see below) are present in the Little Wolf drainage, and at some sites, both populations (and/or possible hybrids) are present. Sites in which flea beetles have been recovered ranged from seasonally moist (e.g. intermittent streams) to dry (e.g. burnt slash piles).

There has been a gradual increase in the number of adult flea beetles recovered in vacuum sampling. The mean number of adults was 2.4/100 plants in 2004 (range, 1–5), 2.4/100 plants in 2005 (range, 1–5) and 7.8/100 plants in 2006 (range, 1–42). The number of beetles collected represents only a small portion of the beetles actually present due to sampling bias (i.e. the sampling technique). Also the number of adults collected may vary due to date, time of day or weather conditions. We consider the Swiss population of the flea beetle to have become established since it has persisted for several years and increased in density, although no dispersal from the original sites has been observed.

## Phenological development

Despite low flea beetle populations, a general indication of the phenological development of the three *L. jacobaeae* populations in Montana can be inferred. The life history of the Oregon low-elevation population appears to be similar to that reported in the literature (Frick and Johnson, 1973; Windig and Vrieling, 1996), with the exception of a longer larval developmental period. Adults emerge from pupation in mid-September. Eggs and first instar larvae were observed in mid-October and early November. During this period, soil tempera-

tures can drop as low as −2.5°C. These temperatures did not seem to adversely affect adults or larvae present at field sites. But by emerging in September, adults may have a reduced period of time for oviposition before the onset of colder temperatures, which may limit the subsequent population. This phenology in Montana is very similar to that reported for the Oregon high-elevation population from Mt. Hood (Markin and Birdsall, 2002). In Montana, the life history of the Oregon high-elevation population differs in that adults emerge in early August, rather than in September. This would be more advantageous to population development, as it extends the oviposition period of *L. jacobaeae*. The life history of the Swiss population in Montana is very similar to that reported by Frick (1971) and Puliafico (2003). Adults have been collected from early August to November. Oviposition occurs approximately 2 weeks after adult emergence. Eggs remain in a semi-diapaused state until spring, and larvae complete their development by late July. The main disadvantage of this life history is that eggs are present in the soil during the driest time of the year and may be subjected to desiccation.

# Conclusions

The tansy ragwort flea beetle appears to be well established in Montana. This is the first report of establishment of this beetle east of the Cascade Mountains of the United States. It is speculated that the Oregon low-elevation population failed in Montana due to low numbers released and phenological incompatibility. The Oregon high-elevation population is well established but may be environmentally limited to moist habitats. The emergence of adults of this population occurs earlier in Montana than in Oregon, giving adults time to lay larger numbers of eggs before winter. The Swiss population also appears to have established but may be less restricted in its habitat requirements, thereby making it a superior control agent. Future research will address the environmental suitability of the two *L. jacobaeae* populations in Montana and their potential impact on tansy ragwort.

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# Factors affecting mass production of *Duosporium yamadanum* in rice grains

**D.M. Macedo, R.W. Barreto and A.W.V. Pomella**

*Summary*

*Duosporium yamadanum* (Matsuura) Tsuda & Ueyama is a pathogenic fungus that attacks purple nutsedge, *Cyperus rotundus*, L. in Brazil. Occasionally, it is found causing severe natural epiphytotics of leaf blight on that host in the field. Exploratory studies have already indicated that this fungus has potential for the development of a mycoherbicide. To help confirm its potential, we studied mass production of inoculum of *D. yamadanum* in solid fermentation using polished rice as the substrate for cultivation of *D. yamadanum*. The effects of the following factors were investigated for their influence on conidial production: water content, length of incubation period before the opening of the plastic bags containing the substrate and addition of supplements to the substrate. Maximum production of conidia was obtained with a water content of 40–60% in the substrate (w/v), with average production at each harvest of $2.5 \times 10^5$ conidia per gram of substrate. Water contents above 60% inhibited growth and sporulation. Opening bags containing the inoculated substrate after 3 to 4 days resulted in the highest levels of sporulation; average of $3.0 \times 10^5$ conidia per gram of substrate. The supplementation of the substrate with calcium carbonate did not significantly increase the sporulation levels compared to controls, whereas the addition of urea or sucrose led to a significant reduction in sporulation; average of $9.0 \times 10^4$ conidia per gram of substrate.

***Keywords***: *Cyperus rotundus*, purple nutsedge, weed biological control, bioherbicide.

## Introduction

Purple nutsedge, *Cyperus rotundus* L., is often considered the world's worst agricultural weed (Holm *et al.*, 1977). Infestations are extremely difficult to control through mechanical methods, and chemical control has either been ineffective or limited by cost, environmental or management problems associated with the most promising products. This weed has been a target by several biological control programmes involving insect (Frick and Quimby, 1977; Frick *et al.*, 1979; Phatak *et al.*, 1987) and fungal (Phatak *et al.*, 1982; Upadhyay *et al.*, 1991; Prakash *et al.*, 1996; Neto, 1997; Okoli *et al.*, 1997; Ribeiro *et al.*, 1997; Kadir *et al.*, 1999, 2000a,b; Rosskopf *et al.*, 1999; Kadir and Charudattan, 2000) natural enemies. Although some experimental results have been particularly promising, no commercial mycoherbicide is available, and there are no classical biological control agents in the pipeline. A combination of methods in an integrated management approach is often mentioned as ideal to minimize the problems associated with intensive chemical control (Bariuan *et al.*, 1999). Although Brazil is known to be outside the centre of origin of *C. rotundus*, a survey carried out in this country revealed a series of fungal pathogens showing clear potential for utilization for the development of a mycoherbicide (Barreto and Evans, 1994, 1996). Among these, the dematiaceous hyphomycete, *Duosporium yamadanum* (Matsuura) Tsuda & Ueyama, was selected as specially promising, as it was capable of spontaneously causing severe leaf blight epihytotics in *C. rotundus* in the field (Barreto and Evans, 1994). A series of intensive studies on the biology and management of *D. yamadanum* was initiated in 1995 by Pomella (1999) and continued by Macedo (2006) and mostly confirmed that this fungus has potential as a biological control agent. Among the most common limitations hampering the development of mycoherbicides are those related to the mass production of abundant, good quality inoculum to be used as the active ingredient. Large-scale production of fungal biological control agents for weed biological control is mainly through techniques of liquid, diphasic or solid fermentation (Jackson *et al.*, 1996). Liquid fermentation is the favoured technique because it is nor-

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mally the most economically viable method (Tebeest and Templeton, 1985). The diphasic method is usually not viable economically because it often involves one growth phase on a medium solidified with agar (Walker, 1980), but this is not necessarily true, as there are cheap alternatives to agar. Finally, solid fermentation utilizes substrates such as grains for fungal colonization and sporulation. This technique is commonly used for small-scale production of fungi that do not sporulate well in liquid media (Pandey, 2003). Pomella (1999) reported good results in mass production of *D. yamadanum* through solid fermentation. Additional investigations were undertaken aimed at perfecting the technique originally developed by Pomella (1999). Investigations on the effects of some parameters on sporulation such as use of nutritional supplements, length of period of incubation before initiation of harvesting and water content in substrate were performed.

# Material and methods

## General conditions for experiments

Our experiments used a selected strain of *D. yamadanum* (RWB 476). The fungus was grown for 7 days in Petri plates containing V8-juice agar, at 25°C, under a light regime of 12 h/day. Three culture discs (diameter, 20 mm) were cut from the margin of actively growing cultures and transferred to flasks containing 100 ml of sterile (autoclaved) liquid medium (200 ml V8-juice diluted with 800 ml of water). Flasks were maintained for 4 days under agitation at 140 rpm. The resulting mycelium was aseptically blended within the remaining medium, and 20 ml of the mycelial suspension was used as seed and transferred to each of a series of polypropylene bags containing 150 g of polished rice + 75 ml of water. The bags with rice were autoclaved before seeding with mycelium of *D. yamadanum*. After seeding, the bag were closed and left in a controlled temperature room at 25°C and a 12 h/day light regime (light from two fluorescent lamps and one NUV BLB 60 W lamp placed 40 cm above the bags) for either 3 days or (in one specific experiment) for a series of five periods of incubation of different lengths. The mass of grains was loosened by gently pressing the bags with the hands to allow for a uniform colonization of the substrate by the fungus. After 3 days, the bags were opened, and a first conidial harvest was performed. Colonized rice grains were then transferred to a flask containing 250 ml of sterile water supplemented with an antibiotic (chloranphenicol at 2.5 ppm) and vigorously stirred with a glass rod. The rice was sieved out of this suspension and placed on aluminium trays (19 × 28 × 2 cm) previously cleaned with 70% alcohol and held at 25 ± 3°C for further periods of conidial production. An interval of 24 h between each harvesting episode was maintained. The quantity of conidia in the remaining suspension was estimated with a haemocytometer. At 24-h intervals, the harvesting procedure was repeated, and conidial production was evaluated. The number of harvests performed varied for different experiments.

## Effects of different levels of water content in the substrate on sporulation of *D. yamadanum*

Pomella (1999) used an arbitrary proportion of 80% water in the substrate with seemingly adequate results. A range of different proportions of water were tested in this experiment, namely 40%, 50%, 60%, 70% and 80% of volume of water per weight of substrate (polished rice grains). Our experimental unit consisted of one plastic bag containing 150 g of rice seeded with *D. yamadanum*. The experiment was arranged in a completely randomized scheme with four repetitions.

## Influence of different supplements added to the substrate on sporulation of *D. yamadanum*

The effect of nutritional supplementation of the basic substrate (polished rice) on sporulation of *D. yamadanum* was investigated by following the general procedure described above. The treatments consisted in supplementing the water added to the basic substrate (polished rice) before autoclaving with either calcium carbonate (1.5 g/l), urea (2.0 g/l) or sucrose (20 g/l). Control consisted of a group of bags containing the basic substrate without any supplement. Evaluation of sporulation was performed as previously described. Nine harvests were done in this experiment, which was arranged in a completely randomized scheme with four repetitions.

## Effects of different lengths of incubation of *D. yamadanum*, before initiation of harvesting, on sporulation

In this experiment, the general procedure described above was followed, but different periods of incubation between seeding the substrate and the first harvest were tested: 3, 4, 5, 6, 7 and 8 days of incubation at the aperture of the recipients (DIAR). The evaluation was performed as described above. The experiment was arranged in a completely randomized scheme with four repetitions.

## Statistical analysis

Variance analysis were performed with the software SAS (version 8.3; Statistical Analysis System, Cary, NC, USA). Conidial production was evaluated by calculations of areas under the curve as described by Madden *et al.* (2007). The assays with an independent quantitative variable were analyzed by comparing areas under the curve of conidial production (AUCCP) obtained for each treatment. Variance analysis of the effects of treatments was performed and compared with Tukey's test at 5% of probability.

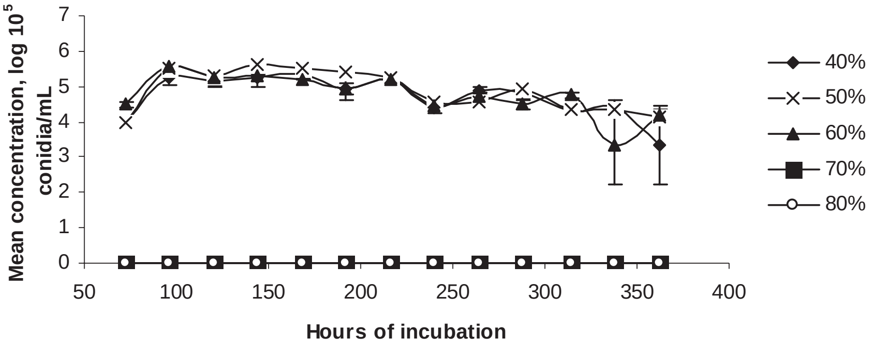


**Figure 1.** Mean concentration of log $10^5$ conidia per millilitre of *Duosporium yamadanum* produced for treatments with different percentages of water added to the substrate (for a total of 13 harvests). Bars represent the standard error.

# Results

## Effects of different levels of water content in the substrate on sporulation of *D. yamadanum*

The highest sporulation levels were obtained with a water content ranging from 40% to 60%. The largest yield of conidia was obtained in the second harvest. A clear reduction in yields was observed on the tenth harvest, that is, at hour 314 (Fig. 1). No statistical difference was observed for total yield obtained for treatments with 40%, 50% and 60% of water. No colonization or sporulation was obtained for the treatments with 70% and 80% of water (Fig. 2).

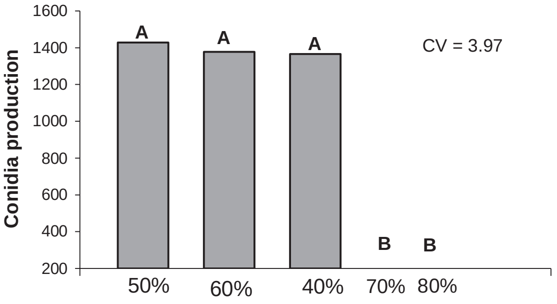


**Figure 2.** Conidial production (AUCCP) for different water percentage on the substrate (mean of four repetitions). Columns with the same letters did not differ statistically under Tukey's test at 5% of probability.

## Influence of different supplements added to the substrate on sporulation of *D. yamadanum*

The highest levels of sporulation were attained for the first harvest in all treatments (Fig. 3). Although the highest level of sporulation was obtained for the treatment where calcium carbonate was added to the substrate, there was no statistical difference between this treatment and the control. Treatments involving supplementation with urea and sucrose led to significantly less sporulation (Fig. 4).

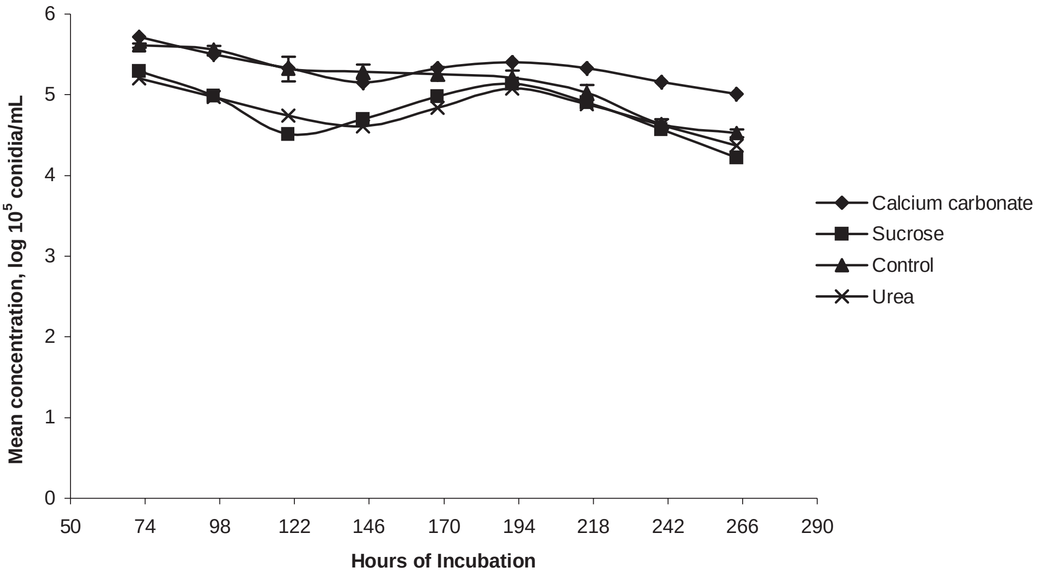


**Figure 3.** Mean concentration of log $10^5$ conidia per millilitre of *Duosporium yamadanum* for substrates supplemented with different substances (total of nine harvests). Bars represent the standard error.

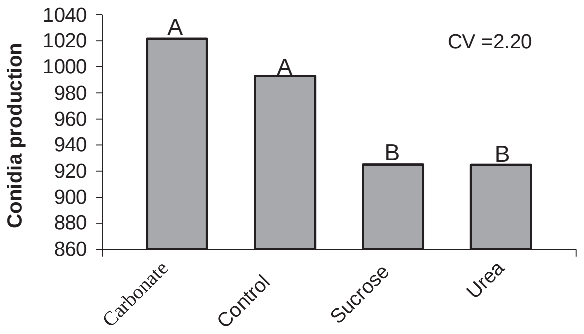


**Figure 4.** Conidial production (AUCCP) for substrates supplemented with different substances (mean of four repetitions). Columns with the same letters did not differ statistically under Tukey's test at 5% of probability.

## Effects of different lengths of incubation of *D. yamadanum*, before initiation of harvesting, on sporulation

Allowing *D. yamadanum* to grow on rice within the bags for 3 or 4 days was shown to be significantly better, in terms of sporulation, than the other periods that were tested. Longer periods of incubation led to a significant reduction in sporulation. Sporulation was maintained for longer periods of time for treatments subjected to shorter periods of incubation and dropped to zero after eight harvests (for 8 days of incubation) and at the last harvest (for 7 days of incubation; Figs. 5 and 6).

# Discussion

Viability of a mycoherbicide depends heavily on the development of an adequate methodology for large-scale production of fungal propagules (Jackson *et al.*, 1996). Each fungus has different requirements for optimal production (quantity and quality), and often minor adjustments in a mass production protocol may have significant impacts on final results. Although almost all commercial mycoherbicides have relied on liquid fermentation, mass production of entomopathogens has generally relied on solid fermentation, particularly based on grains, such as rice, as a substrate (Wyss *et al.*, 2001; Tarocco *et al.*, 2005).

Preliminary attempts by Pomella (1999) to mass produce *D. yamadanum* in liquid media failed to yield any sporulation. Later, the same author demonstrated that solid fermentation might offer an adequate alternative for mass production. The protocol described by Pomella (1999) for this purpose is in contrast to the present study in some respects. For instance, water content in the substrate of 40%, 50% and 60% gave better sporulation compared to 70% to 80% found to be best by Pomella (1999). In the present study, higher levels of water content led to an inadequate consistency of the substrate. In high water-content treatments, grains became too soft and water soaked and lack of aeration within the substrate mass probably did not allow proper colonization of the substrate and sporulation of the fungus. Other differences between the two production protocols may explain the discrepancies between observations made in this work and those made by Pomella (1999). In the latter, seeding of the medium was done with culture disks, harvests were initiated much later (13 days) and the plastic bags were not completely sealed but had a cotton plug closing the bag's openings. This might have allowed a progressive dehydration of the substrate generating conditions that were less favourable for the fungal growth than those provided by the set of conditions adopted in the present study.

Of particular interest was the persistence of sporulation on colonized rice grains observed in this study. After 13 harvesting episodes, conidia were still relatively abundant for the best treatments, despite the progressive decline in sporulation. Our utilization of blended

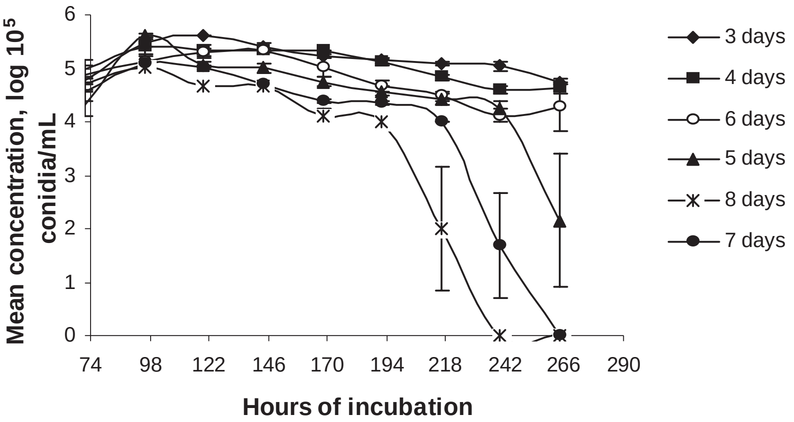


**Figure 5.** Mean concentration of log $10^5$ conidia per millilitre of *Duosporium yamadanum* for different periods of incubation within sealed plastic bags before initiation of harvesting. Bars represent the standard error.

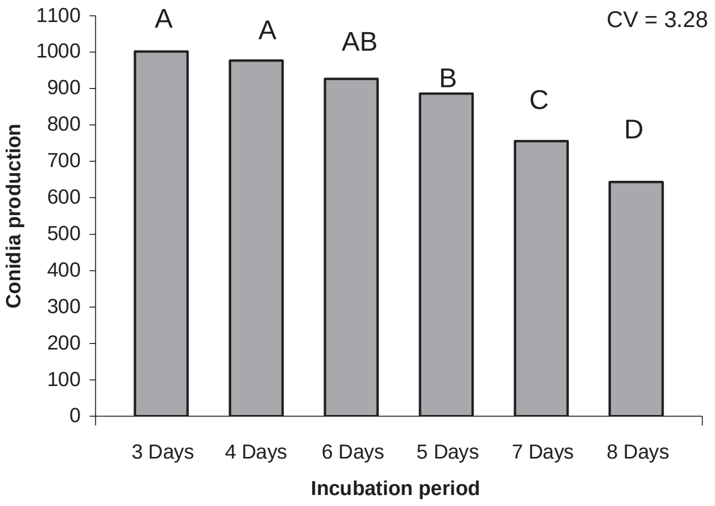


**Figure 6.** Conidial production (AUCCP) for different periods of incubation within sealed plastic bags before initiation of harvesting (mean of four repetitions). Columns with the same letters did not differ statistically under Tukey's test at 5% of probability.

mycelium as seed allowed for a quick colonization of the substrate, shortening considerably the process of conidium production for this fungus. The period of spore production and harvest number was increased.

The levels of moisture that are more appropriate for mass production of fungi vary from species to species. For example, the ideal moisture content in the substrate was shown to be 30% to 40 % for *Metarhizium anisopliae* (Metsch) Sorok. var. *acridum* (Arzumanov *et al.*, 2005) and also for *Penicillium oxalicum* Currie & Thom (Larena *et al.*, 2002), whereas for *Mucor bacilliformis* Hesselt, the ideal is 90% (Lareo *et al.*, 2006). The long period of incubation within the bags adopted in Pomella's protocol was shown to be unnecessary. Pomella waited for a thorough and visible colonization of the rice mass before opening the bags and starting the conidial harvest to avoid problems with contamination. However, using the techniques described in this paper, contamination was not a problem and before *D. yamadanum* colonies are visible with naked eye, colonization is well advanced. Keeping the bags closed for longer periods was shown to be harmful for sporulation, perhaps because it leads to stress such as reduced exchange of gases, lack of oxygen, poor heat dissipation or others.

Supplementing the substrate with urea or sucrose is known to increase sporulation for many fungi, but in the case of *D. yamadanum*, it was clearly harmful. The addition of calcium carbonate resulted in a statistically negligible increase in sporulation. Higher concentrations of calcium carbonate might have a more significant effect on sporulation, an aspect deserving further investigation.

The results obtained in this study provide improvement on the protocol for mass production proposed by Pomella (1999).

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# Biological control of tansy ragwort (*Senecio jacobaeae*, L.) by the cinnabar moth, *Tyria jacobaeae* (CL) (*Lepidoptera*: *Arctiidae*), in the northern Rocky Mountains

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*Summary*

The control of tansy ragwort on the coast of western North America is a major success story for weed biological control. However, tansy ragwort is still expanding into the colder interior regions of the Pacific Northwest of the United States where previous efforts to establish the same complex of agents have failed. We have successfully established one of the agents, the cinnabar moth, *Tyria jacobaeae* L., on a major new tansy ragwort infestation in the mountains of northwestern Montana. The cinnabar moth is still expanding its range, but in the areas where first released, it has given excellent control, having eliminated tansy ragwort as a visible component in the forest ecosystem while not impacting native *Senecio* species. Although establishment in other areas has been slower, we predict that we will eventually control tansy ragwort over most of its range in the northern Rocky Mountains of the United States.

***Keywords***: tansy ragwort, *Senecio jacobaea*, cinnabar moth, *Tyria jacobaeae*.

## Introduction

In the Pacific Northwest corner of the United States, tansy ragwort, *Senecio jacobaea* L., (*Asteraceae*), an introduced European forb, is an invasive weed in pastures, native meadows and open forests (Coombs *et al.*, 1991, 1999). It is a particularly serious problem for grazing livestock because it contains toxic alkaloids. Along the Pacific Northwest coast in the 1960s and 1970s, a USDA-ARS program successfully established three biological control agents and resulted in one of the most successful biological control weed programs in North America (Turner and McEvoy, 1995; Coombs *et al.*, 1996, 2004; Julien and Griffith, 1998). Tansy ragwort, however, is still spreading east of the Cascade Mountains into eastern Oregon, Washington and northern Idaho.

In 1994, a wild fire burned 6100 ha of fir and pine forests in a mountainous area straddling the boundary between Lincoln and Flathead Counties in northwestern Montana. Tansy ragwort was probably already present, but after the fire, an explosive flush of new plants occurred, which was first noticed in 1996. Management plans were immediately initiated with the goal of herbicide spraying to begin in 1997. In attempting to obtain funding for the program, it was necessary to develop an integrated management strategy, and biological control was added as an afterthought.

Initially, we felt the three biological control agents used on the west coast would fail in Montana, but the funding provided the chance to investigate why these agents had not established previously when released east of the Cascade Mountains. Our preliminary studies showed that all three agents would establish, but in Montana, the cinnabar moth, *Tyria jacobaeae* (L.) (Lepidoptera: Arctiidae) was particularly suitable. This paper describes the success we observed in subsequent years to the release of this moth.

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# Materials and methods

## Source of cinnabar moths

The first colonies of cinnabar moths, obtained from Oregon in 1996 from the Willamette Valley and along the coast near Florence, were used for preliminary non-target host studies. Additional shipments from the Willamette Valley in 1997 were used for three field-cage releases within the area burned by the forest fire in Flathead County in Montana. In 1998, a new moth population was located near Mount Hood at 1300-m elevation in the Cascade Mountains of Oregon in a forested site with a heavy winter snow cover, similar to northwestern Montana. From 1998 to 2000, egg masses from the Mount Hood population were shipped to Bozeman, sterilized by soaking in a 0.1% sodium hypochloride for 5 min and rinsed in running water to eliminate bacterial or viral contaminants. After hatching, first-instar larvae were held, ten individuals to a Petri dish, for 10 to 14 days, fed tansy ragwort leaves treated with 100 ppm of the fungicide Benomyl (Benlate sp. fungicide, DuPont Agricultural Products, Willmington, DE, USA) to eliminate microsporidium that were reported to be present in the west coast cinnabar moth populations (Bucher and Harris, 1961; Hawkes, 1973). If any larvae died, the entire batch was discarded. Larvae that reached third instars without mortality were used for subsequent studies in the laboratory and for field release in 1998 to 2000.

## Non-target plant testing

Initially, there were questions concerning using the cinnabar moth because, in early laboratory testing, it fed on several North American *Senecio* sp. (Bucher and Harris, 1961) and had been reported attacking a field population of *Senecio triangularis* Hook in Oregon (Diehl and McEvoy, 1989). It was therefore necessary to determine whether Montana varieties of *S. triangularis* might also be at risk. In 1996, no-choice feeding tests using third-instar larvae in Petri dishes offering either Montana *S. triangularis* or tansy ragwort leaves were set up, and the rate of development, survival and weight of any pupae produced was determined. In 1997 and 1998, the tests were repeated but expanded to look at other species of *Senecio* found in the Montana tansy ragwort area. Those species were *Senecio* (sym: *Packera) pseudaureus* Rydb., *Senecio hydrophilus* Nutt., *Senecio integerrimus* Nutt. and *Senecio* (sym: *Packera*) *canus* Hook (Hanson, 2000). When these tests indicated that *S. pseudaureus* could be fed on, the first field releases in 1997 and 1998 (of 300 larvae each) were made in 2 × 4 m field cages (three each year) containing intermixed *S. pseudaureus* and tansy ragwort plants.

## Open field release

By 1999, studies indicated that the cinnabar moth was not a threat to native *Senecio*, and the rapid population increases in cages indicated it might be a useful biological control agent for Montana. The original six cages were removed that year so the moths could disperse naturally, and we began a program of additional releases to spread them as rapidly as possible through the remaining tansy ragwort area.

The tansy ragwort infestation in Montana occurs in two counties, Flathead County and Lincoln County, each differing environmentally and in land ownership. In Flathead County, the infestation was restricted to National Forest lands in the eastern half of a 4000 ha area of remote rugged fir and pine forest (elevation, 1350–1560 m) that was burned in a wildfire in 1994 and subsequently salvage logged. Initially, the tansy ragwort infestation was thought to cover only 100 ha, and the program was aimed at controlling and, if possible, eradicating the infestation using herbicides. By 1997, additional surveys indicated that there were at least 500 ha to be sprayed. There were also numerous small environmentally sensitive sites located close to water where herbicide was not allowed to be used, and these were used for the initial biological control studies. Besides the six caged releases in 1997 and 1998, uncaged releases of 300 larvae each at eight new field sites in the burned area and three in the surrounding unburned forest were made in 1999.

In 2000, the program was expanded westward into the Little Wolf Creek drainage in Lincoln County where the remaining third of the area burned by the wildfire also contained dense stands of unsprayed tansy ragwort. This area was primarily the property of a timber company, which thought chemical control was not economically justifiable. In 2000 and 2001, 12 releases of 300 early instar larvae, collected from the now well-established populations in Flathead County, were made. From 2002 to 2004, emphasis shifted to a third area approximately 12 km to the southwest of Little Wolf Creek in the vicinity of Island Lake, a small mountain lake. The land ownership here was a mixture of National Forest, private timber company and private ranches. The area differed from the first two targeted areas, as it was unburned and consisted of an 8000-ha mosaic of different aged fir, pine and larch forests and open meadows. The area was extensively disturbed by heavy grazing and logging. Tansy ragwort was concentrated in the more open and disturbed sites and was as abundant and dense as in the burned area. Seven releases of 300 early instar larvae from the Flathead County population were made in 2002.

## Monitoring impact

During the three caged releases made in 1997, and repeated in 1998, all tansy ragwort plants in the cages were recorded as either seedlings, rosettes (would not bolt that year) or mature plants that had bolted. These gave estimations of populations within the six caged areas and were compared to populations at six similar

but uncaged areas between 50 and 100 m away, at which no releases were made. In 1999, when the cages were removed, a permanent marker was placed at the centre of each of these 12 study sites. At 1 m radius out, all tansy plants found within four 25-cm$^2$ quadrats were counted to determine site density. Monitoring through the remainder of this program continued at the six initial caged release sites and the six uncaged areas. Within a few years, all uncaged areas had been inundated by the cinnabar moth so all data has been combined for an average of the 12 study sites.

When the program changed from research in 1999 to an operational program in which we were trying to redistribute the cinnabar moth as rapidly as possible, no further detailed monitoring was conducted, although all new releases were marked. These sites were visited annually, and visual estimates were made of the abundance of tansy ragwort and cinnabar moth larvae in mid- to late July when the plants were in flower and the larvae were most active and visible. Larvae population estimates were made by walking a 50-m transect in 5 min while counting or estimating the number of larvae seen feeding on the flower heads and, later in the study, on any surviving rosettes.

# Results

## Non-target feeding

Laboratory feeding tests showed that *S. triangularis* from Montana was an unsuitable host (Table 1). Feeding occurred in starvation tests, but development was much slower and produced only a few small pupae. Even poorer development was seen on *S. hydrophilus*, *S. integerrimus* and *S. canus*. Subsequent observations in the field on natural populations of *S. triangularis* and *S. hydrophilus* intermixed with tansy ragwort at five locations in Flathead County showed that, even during the peak population of the cinnabar moth and its collapse after the elimination of the tansy ragwort, both species were totally ignored. Why *S. triangularis* should be fed on in the field in Oregon but not in Montana has not been resolved.

By contrast, *S. pseudaureus* in laboratory no-choice feeding tests supported almost normal development of larvae, although the larvae would not feed on it in choice tests when tansy ragwort was also available. Subsequent field observations showed that, during the first 2 or 3 years of the cinnabar moth build up, *S. pseudaureus* was ignored. However, when the supply of tansy ragwort was exhausted, females would occasionally lay egg masses on *S. pseudaureus*. The resulting larvae skeletonized the leaves that contained the egg mass and then disappeared. Furthermore, if late-instar larvae totally consumed adjacent tansy ragwort plants and dispersed in search of food, they occasionally fed on *S. pseudaureus*. This minor feeding was observed for a year or two until the tansy ragwort had disappeared, and when the cinnabar moth population collapsed, feeding on *S. pseudaureus* ceased. At no time after the disappearance of tansy ragwort in 2003 have any cinnabar moths been found utilizing *S. pseudaureus* as a permanent host. *S. pseudaureus* in Montana therefore will not support a permanent population of cinnabar moths and suffers only temporary attack when adjacent tansy plants are stripped of foliage. The potential for it to attack other *Senecio*'s exists, however, so this moth should not be released in new areas without preliminary host testing of local *Senecio*.

## Establishment of the cinnabar moth

Of the six original cage releases, five built up populations that, by the second year, were causing 50%

**Table 1.** Comparison of survival rate and development times for larvae and pupal weight of the cinnabar moth raised on tansy ragwort, *Senecio jacobaeae*, and other species of *Senecio* native to Montana.

| Species | Larvae no. | Pupae no. | Pupate % | Days to pupate | Pupae weight (g) |
|---|---|---|---|---|---|
| 1997 | | | | | |
| *Senecio jacobaea* | 26 | 24 | 92.3(a)[a] | 22.6(a)[a] | 0.122(a)[a] |
| *Senecio pseudaureus* | 24 | 16 | 66.7(b) | 25.0(a) | 0.110(a) |
| *Senecio hydrophilus* | 28 | 16 | 57.1(b) | 32.6(a,b) | 0.070(b) |
| *Senecio triangularis* | 21 | 2 | 9.5(c) | 46.0(b) | 0.070(b) |
| *Senecio integerrimus* | 22 | 0 | 0.0 | 0.0 | 0.000 |
| *Senecio canus* | 25 | 0 | 0.0 | 0.0 | 0.000 |
| 1998 | | | | | |
| *Senecio jacobaea* | 43 | 34 | 90.5(a) | 20.4(a) | 0.130(a) |
| *Senecio pseudaureus* | 42 | 38 | 79.1(a) | 21.9(a) | 0.110(a) |
| *Senecio hydrophilus* | 29 | 22 | 75.9(a) | 28.2(b) | 0.080(b) |
| *Senecio triangularis* | 21 | 2 | 9.5(b) | 39.5(b,c) | 0.080(b) |
| *Senecio integerrimus* | 30 | 1 | 3.3(b) | 29.0(c) | 0.090(a,b) |
| *Senecio canus* | 33 | 1 | 4.4(b) | 40.0(c) | 0.090(b,b) |

[a] Each year, grouping is compared based on Tukey HSD at 0.05 level.

defoliation. Of the eight additional open releases, all established and spread and, by 2004, had reached 95% of the infested area. In Flathead County, the cinnabar moth population collapsed after the disappearance of the tansy ragwort around 2003. While plants continue to sprout from the soil seed bank and may escape detection while small rosettes, within a year of flowering, they are usually found by the moth and destroyed. This pattern of low numbers of cinnabar moth and low plant number equilibrium has been observed in Oregon for the last 20 years and we expect is now the situation in Flathead County. The exception is a small (less than a quarter hectare) site in which a dense stand of tansy ragwort has, so far, escaped attack in the moist bottom of a narrow valley.

In the Little Wolf area, Lincoln County, six of seven releases, which were made on a hillside, established but population build up was slower than in the Flathead County sites. A large amount of tansy ragwort, resulting from the lack of a spray program, is present. Consequently, it will take longer for the cinnabar moth population to reach a level capable of overwhelming this huge biomass, a problem not encountered in Flathead County where the majority of the tansy ragwort biomass had been eliminated by herbicide spraying. By contrast, the five releases made within 30 m of Little Wolf Creek failed. It is presumed that these and the failure in Flathead County are due to micro-climatic effects restricted to these narrow mountain valley bottoms.

In Flathead County, establishment occurred at three sites in unburned forest, but our initial six releases, of 300 larvae each, in the unburned area around Island Lake failed. In 2004, releases of between 1000 and 2000 late instar larvae were made at ten sites around the lake. By 2005, most of these releases established. By 2006, five had expanded, and they were totally defoliating the tansy in areas ranging from 0.5 to 5 ha; three had low populations that were not causing significant defoliation, and two appeared to have failed. We have no explanation why small releases of early instar larvae failed, but large releases of late instar larvae gave rise to rapidly expanding populations.

## Impact

Tansy ragwort density at the 12 original sites in Flathead County remained constant for the first 3 or 4 years, while the populations of the cinnabar moth built up. They then declined to a low in 2003 when tansy ragwort was almost undetectable (Fig. 1). The cinnabar moth population then collapsed due to the lack of food. The tansy ragwort re-sprouted from the seed bank, and the peak in 2004 represents only new seedlings or very small rosettes. However, the cinnabar moth soon reappeared at all sites and suppressed the plant. Today, the tansy ragwort and the cinnabar moth appear to be in equilibrium, maintaining the plant population at a much suppressed level. The major visible impact of our program has been the almost total disappearance of flowering tansy ragwort plants since 2002. The Little Wolf and Island Lake areas in Lincoln County now have well-developed moth populations, and in many areas, they have caused the demise of mature plants. We predict that these populations will continue to increase and spread and that they will reduce tansy ragwort to levels comparable to those in Flathead County.

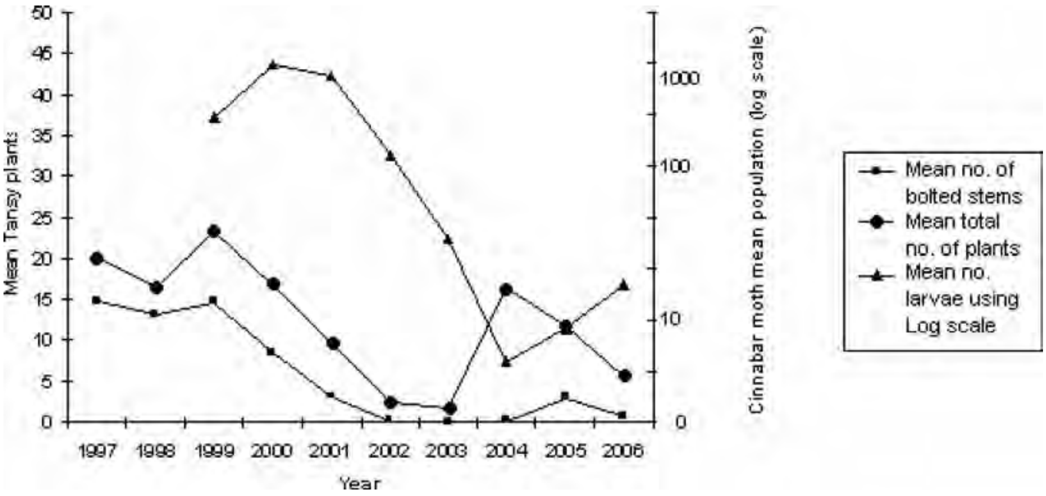


**Figure 1.** Density of tansy ragwort, *Senecio jacobaeae,* showing the total numbers of plants and the numbers that were mature and bolted. Data are the mean of 12 permanent plots at Flathead County, MT. The estimated numbers of late-instar larvae of the cinnabar moth, *Tyria jacobaeae*, counted in 5 min along a 50-m transect at the same 12 sites. No estimate for 1997 or 1999 since the cinnabar moth was confined in field cages.

# Discussion

## Comparison of effectiveness between Montana and Oregon

The success of the cinnabar moth in Montana raises the question why this insect fails to control tansy ragwort along the west coast (van der Meijden, 1979; Myers, 1980; Crawley and Gillman, 1989). This may be due to climate differences affecting the phenology of the plant. In Oregon, the cinnabar moth was often observed defoliating large mature plants. However, the plant compensated with re-growth during the following mild, wet fall and winter (Hawkes, 1981; Cox and McEvoy, 1983; Turner and McEvoy, 1995). In Montana, plants that survive defoliation do not recover noticeably before the onset of cold weather in October and permanent snow cover in November (Fig. 2). Surviving plants emerge the following spring as small rosettes that seldom flower. Several consecutive years of cinnabar moth feeding on even the strongest plants in Montana continue to reduce them in size until they are eventually killed. It is interesting that the only other location where the cinnabar moth is credited with controlling tansy ragwort is the eastern Maritime Provinces of Canada (Harris *et al.*, 1973, 1978), an area similar to Montana with a long cold winter and snow cover that probably protect the over wintering pupae in the soil from freezing (Fig. 2).

## Future of the cinnabar moth in Montana

On the west coast, the cinnabar moth populations are affected by severe diseases (Bucher and Harris, 1961; Hawkes, 1973). We took great efforts to eliminate diseases from the populations that we introduced to Montana, and our monitoring there has detected no sign of disease that could limit the cinnabar moth's effectiveness.

To counter this possibility, we are continuing our effort to establish the tansy ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse), in those areas where the cinnabar moth has not established. Hopefully, flea beetle colonies will be numerous enough that, if the cinnabar moth population eventually collapses, they will be ready to replace it (see Littlefield *et al.*, this volume).

## An integrated control program

The rapid success obtained in eliminating tansy ragwort in Flathead County was due to a combination of herbicide application and biological control. There was an intensive herbicide spray program between 1997 and 1999 that probably killed 99% of mature, flowering plants. The only unsprayed tansy ragwort was in the buffers around springs, moist seeps or riparian zones, where spraying was prohibited and which was used for the biological control study. However, after spraying, a flush of seedlings was observed, and by 2001 and 2002, flowering plants began to reappear as the replacement generation matured. At this time, the cinnabar moth population was well established at our research sites, spreading rapidly through the surrounding tansy ragwort area and, within a few years, eliminated the need for additional chemical treatments. Chemical treatment of tansy in Flathead County is now limited to roadside spraying of any isolated plants found to prevent their

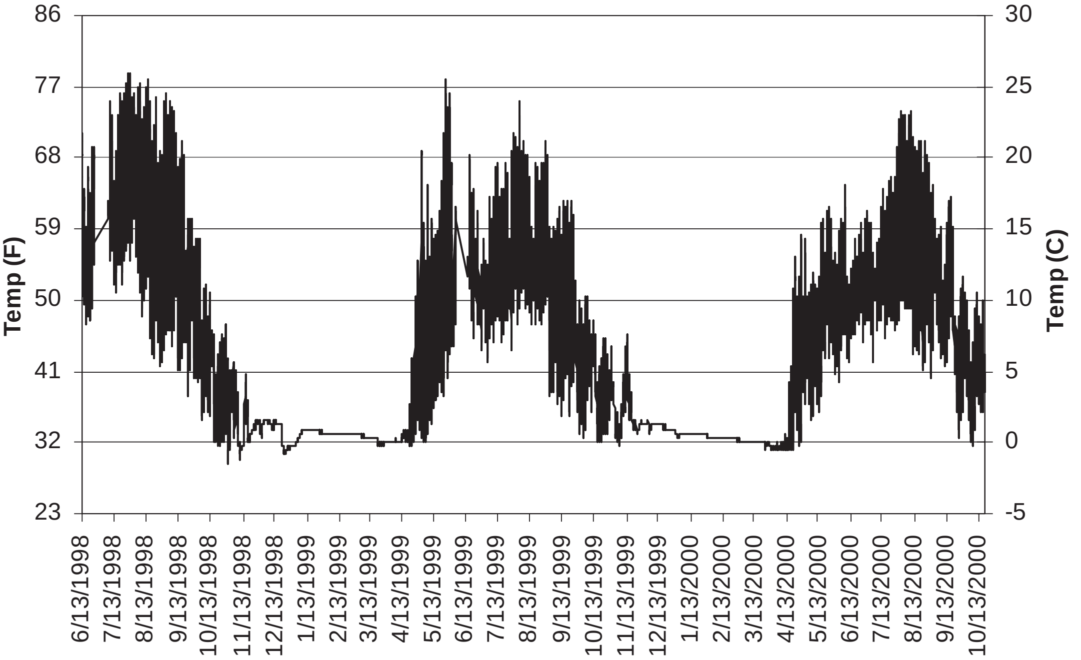


**Figure 2.** Soil temperatures 2 cm below the surface at site 1 in the Flathead National Forest. *Relatively flat lines* from November to April indicate snow cover.

seeds from being carried out of the area and the spraying of isolated satellite population, as they are found in outlying areas.

The successful control in Flathead County, although unplanned, is an excellent example of how an integrated control program for a new weed can be implemented. In this case, herbicides contained a new infestation and suppress seed production in the core area long enough for the biological control agents to establish and build up populations capable of dispersing and overwhelming the suppressed population.

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# Establishment, spread and initial impacts of *Gratiana boliviana* (*Chrysomelidae*) on *Solanum viarum* in Florida

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*Summary*

*Solanum viarum* Dunal (Solanaceae) is an invasive perennial shrub in southeastern USA. Native to South America, it was first found in Florida in 1988, and it has already invaded more than 400,000 ha of grasslands and conservation areas in 11 states. Currently recommended control tactics for this weed in pastures are based on herbicide applications combined with mechanical (mowing) practices. These control tactics provide a temporary solution and can cost as much as $188/ha for dense infestations of the weed. A biological control project against *S. viarum* was initiated in 1997. After 3 years of intensive host-specificity testing, the South American leaf beetle *Gratiana boliviana* was approved for field release by the United States Department of Agriculture (USDA)-Animal and Plant Health Inspection Service (APHIS)-Plant Protection and Quarantine (PPQ) in 2003, and its release in Florida began in summer 2003. Up to now, approximately 120,000 beetles have been released in 25 counties in Florida. The beetles established at virtually all the release sites in Florida. Beetle dispersal has been based on plant availability with annual dispersal from 1.6 to 16 km/year from the release sites. Initial impacts of the beetles range from 30% to 100% plant defoliation. The fruit production declined from 40 to 55 fruits per plant in summer 2003, when beetles were released, to zero or a few deformed fruits (one to four per plant) 2 years post release in five of the release sites monitored. Mass rearing, field release and post-release evaluation of *G. boliviana* and the target plant will continue during 2008.

***Keywords***: invasive plant, weed biological control, monitoring.

## Introduction

*Solanum viarum* Dunal (Solanaceae) is a perennial shrub from South America that has been spreading throughout Florida at an alarming rate during the last two decades. The pastureland infested in 1992 was estimated in approximately 60,000 ha (Mullahey *et al.*, 1993), and this infested area increased to more than 300,000 ha in 1995–1996 (Mullahey *et al.*, 1997). Currently, the infested area is estimated at more than 400,000 ha (Medal *et al.*, 2004; Medal, 2005). *S. viarum* was first reported in the United States in Glades County, FL, in 1988 (Coile, 1993; Mullahey and Colvin, 1993). This weed also is present in Alabama, Arkansas,

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Georgia, Louisiana, Mississippi, North Carolina, Pennsylvania, South Carolina, Tennessee, Texas and Puerto Rico (Bryson and Byrd, 1996; Dowler, 1996, Mullahey *et al.*, 1997; Medal *et al.*, 2003). However, infestations in these states have still not reached high levels. The potential range of *S. viarum* in the United States can be extended even further based on studies of the effects of temperature and photoperiod conducted by Patterson (1996) in controlled environmental chambers. This invasive exotic weed was placed on the Florida and Federal Noxious Weed Lists in 1995.

In addition to its invasion of pasture lands and reduction of cattle carrying capacity (Mullahey *et al.*, 1993), *S. viarum* is a host of at least six viruses that affect vegetable crops including tomato, tobacco and pepper (McGovern *et al.*, 1994a,b, 1996). Furthermore, it is also an alternate host for agricultural pests such as the Colorado potato beetle, *Stilodes* (=*Leptinotarsa*) *decemlineata* (Say) (Coleoptera: Chrysomelidae), a major defoliating insect pest of potato in North America; tomato hornworm *Manduca quinquemaculata* (Haworth) (Lepidoptera: Sphingidae), a major pest of tomato and tobacco plants; and the silverleaf whitefly *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae), one of the most troublesome insect pests worldwide of many field and vegetable crops (Habeck *et al.*, 1996; Medal *et al.*, 1999). Although it is very difficult to determine the real (direct and indirect) economic losses due to this invasive weed, Mullahey *et al.* (1996) estimated the annual production loss to Florida ranchers was US$11 million in 1993.

Native to southern Brazil, Paraguay, northeastern Argentina and Uruguay (Nee, 1991), *S. viarum* has spread into other parts of South and Central America including Mexico, Nicaragua, Honduras and Costa Rica (J. Medal, personal communication). This weed also has spread into other regions including the Caribbean (confirmed in Puerto Rico), Africa, India, Nepal and China (Chandra and Srivastava, 1978; Coile, 1993). The rapid spread in Florida can be partially attributed to the high reproductive potential (Akanda *et al.*, 1996; Pereira *et al.*, 1997) and effective seed dispersal by cattle and wildlife, such as deer, feral hogs, raccoons and birds that feed on the fruits (Mullahey *et al.*, 1993; Bryson *et al.*, 1995; Brown *et al.*, 1996).

One *S. viarum* plant can produce on average from 100 to 160 fruits and 41,000 to 50,000 seeds with a germination rate of at least 75% (Mullahey *et al.*, 1993; Pereira *et al.*, 1997). The extent of the infestation is increasing rapidly in the United States, making this a national rather than just a Florida problem.

Current management practices for *S. viarum* in Florida are based on herbicide applications combined with mechanical (mowing) practices (Mislevy *et al.*, 1996, 1997; Sturgis and Colvin, 1996; Akanda *et al.*, 1997). These control tactics provide temporary weed suppression at an estimated cost of US$185/ha to control dense infestations of *S. viarum* (Mullahey *et al.*, 1996). In addition to being expensive, the application of herbicides is not always feasible in rough terrain or inaccessible areas.

A biological control project on this highly invasive non-native weed was initiated in January 1997 by the University of Florida in collaboration with the Universidade Estadual Paulista, Jaboticabal campus, Brazil; Universidade Federal do Paraná in Curitiba, Brazil; Universidade Regional de Blumenau, Santa Catarina state, Brazil; Universidade Centro-Oeste, in Irati, Paraná state, Brazil; Instituto Nacional de Tecnología Agropecuaria (INTA-Cerro Azul), Misiones province, Argentina; and the United States Department of Agriculture (USDA)-Agricultural Research Service (ARS) Biological control laboratory in Hurlingham, Buenos Aires province, Argentina. Foreign explorations in South America identified several insects as potential biological control agents of *S. viarum* including three leaf beetles, *Gratiana boliviana* Spaeth, *Metriona elatior* Klug and *Gratiana graminea* Klug (Chrysomelidae) and the flower-bud weevil *Anthonomus tenebrosus* (Boheman) (Curculionidae). These potential agents were initially selected for screening because of the extensive foliage/flower bud plant damage attributed to these beetles in their native range (Medal *et al.*, 1996, 1999, 2006). Two other promising biological control candidates that are currently undergoing open-field host-specificity tests in Brazil are the leaf beetle *Platyphora* sp. and a flea beetle (Chrysomelidae) (H. Medal, unpublished data).

*G. boliviana* was approved for field release in the United States by USDA-Animal and Plant Health Inspection Service (APHIS)-Plant Protection and Quarantine (PPQ) in May 2003. A high level of specificity and significant defoliation of *S. viarum* was indicated in host-specificity tests (Gandolfo *et al.*, 1999, 2007; Medal *et al.*, 2002, 2004). Field releases of *G. boliviana* in the United States began in May 2003. Requests for field releases of the leaf-feeder beetles *M. elatior* and *G. graminea* in the United States were submitted to TAG (Technical Advisory Group for Biological Control Agents of Weeds) in September and October 2006, respectively.

## Release of *G. boliviana* in Florida

A total of 120,000 beetles have been released in 25 Florida counties since the summer of 2003. Florida counties where beetles have been released are shown in Fig. 1. The number of beetles released at each location varied from 30 to 2000 based on beetle availability and density of the *S. viarum* infestation.

A new release technique was used for the first time in the *S. viarum* biological control project. *S. viarum* plants that were infested with beetles (approximately 100 per plant) in the greenhouse at the Southwest Florida rearing facility in Immokalee were taken to the field and transplanted in October 2005 and August 2006 in

**Figure 1.** Florida counties (*dark*) where *Gratiana boliviana* have been released during the period 2003–2007.

Lee County, Florida. This has proven to be an efficient technique with less labor involved for insect field release. Beetle releases have been made both on private and public lands. The *G. boliviana* demand by cattle ranchers for field release each year exceeded the beetle production by our team of collaborators. We plan to increase the beetle production in 2007 by establishing field insectaries at different locations in Florida. Cattle ranchers interested in obtaining beetles for release in their farms are being provided with adult beetles, and we are conducting monthly or bimonthly post-release evaluations on the dispersion of the beetles, on the extent of the feeding damage and changes in the beetle population at selected release sites. The post-release evaluations also include observations on possible non-target effects on closely related plant species growing in the release area and on the regeneration of native plant species and/or improved pastures that have been displaced by the *S. viarum* plants.

## Post-release evaluations of *G. boliviana* in Florida

Evaluation of the feeding effects of the beetles on *S. viarum* plants (percent defoliation, fruit production) and number of beetles on plants began in the summer of 2003–2004 in Polk and Okeechobee counties, in St. Lucie and Okeechobee counties and in Collier and Hendry counties. Monitoring also was initiated at the Eagle Creek Conservation area in Orange County by K. Peterman (Environmental Scientist) and J. Medal. For the post-release evaluation in Polk County, where approximately 1000 beetles were released in August

2003, 20 marked plants within 100 m of the initial release site have been thoroughly examined every 2 to 3 months since the summer of 2003. The estimated (visual) defoliation increased on average from 46% (December 2003) to 94% (December 2004), and it was directly associated with the increase in number of adults and immature beetles observed on the plants during the same period, except from August to December 2004 when the number of beetles decreased. In 2005, the plant defoliation was high on average from 69% to 96%. At least half of the 20 marked plants were unable to regrow after complete defoliation by the beetles in the previous year and also due to the competition by other plant species. The number of *S. viarum* fruits produced per plant defoliated by the beetles has significantly decreased with none or very few small fruits compared with the large number of fruit (40–55) observed during the summer of 2003 at the time the beetles were released. Most of the plants on the 4-ha release site have been replaced by other plant species including bahiagrass (*Paspalum notatum* Flueggé), *Rubus* sp., dayflower (*Commelina diffusa* Burm), Caesar weed (*Urena lobata* L.), air-potato (*Dioscorea bulbifera* L.), roadside flatsedge (*Cyperus sphacelatus*, Rottb.), oak (*Quercus* sp.) and other herbaceous vegetation. The estimated *S. viarum* density at the release area (4 ha) at the end of November 2005 was only 5–10%, which is significantly lower than the initial population density (80% to 90%) that was observed in the summer 2003 before the beetles were released. The relatively low number (<100) of beetles recorded on the 20 marked *S. viarum* plants on each monitoring date in 2005 can be attributed to beetle dispersal to *S. viarum* plants as far as 1600 m away (September 2005) from the initial release site. Dispersal of the beetles was associated with the low availability of foliage on the *S. viarum* plants at the release site caused by extensive beetle defoliation during the previous growing season. Dispersal ability of the beetles at five of the Florida release sites ranged from 1.6 to 16 km/year. After 3 years post-release at the Polk County site, beetle defoliation is having a great impact. Fruit production has declined to one to five deformed or no fruits per plant if the beetles start feeding on the plants before fruit formation. Follow-up studies include observations on possible non-target effects on closely related plant species growing in the release area. To date, no non-target effects (J. Medal, personal communication) have been observed even on plants in the same genus such as the non-natives red soda apple (*Solanum capsicoides* All.), wetland nightshade (*Solanum tampicense* Dunal) and turkey berry (*Solanum torvum* Sw.) that are growing intermixed with or in close proximity to *S. viarum*.

## Conclusion

Post-release evaluations of the South-American leaf-beetle *G. boliviana*, first biological control agent whose releases in Florida began in summer 2003 against the invasive non-native spiny shrub *S. viarum*, have indicated an extensive weed defoliation and reduction of fruit production in five of the release sites monitored. The beetle established at almost all the release sites and is spreading to adjacent weed-infested areas. Field observations also confirmed the specificity of the beetle on the target weed, and to date, no non-target effects have been observed even on plants closely related.

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# Dissemination and impacts of the fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *miconiae*, on the invasive alien tree, *Miconia calvescens*, in Tahiti (South Pacific)

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*Summary*

Long-term monitoring of biological control agents in their areas of introduction is essential to assess their effectiveness. There is a need to monitor and evaluate agent dispersal and impacts so that the degree of success can be quantified or reasons for failure can be clearly understood. A fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *miconiae* Killgore & L. Sugiyama (Melanconiales, Coelomycetes, Deuteromycetinae), found in Brazil in 1997 was released in the tropical oceanic island of Tahiti (Society Islands, French Polynesia, South Pacific) to control miconia, *Miconia calvescens* DC (Melastomataceae), a small tree native to Tropical America, which has invaded native rainforests. The plant pathogen, proven to be highly specific to miconia, causes leaf spots, defoliation and eventually death of young seedlings in laboratory conditions. Two permanent plots in Tahiti (Taravao Plateau and Lake Vaihiria) were monitored for a period of 6 years to assess the pathogen's dispersal and impacts on miconia in the wild. Leaf spots were observed approximately 30 days after inoculation. Percentage of infected plants reached 100% after 3 months, and between 90% and 99% of leaves were infected. Subsequent re-infection occurred after 3 months at Vaihiria and 18 months at Taravao. Mortality rate for monitored plants was 15% and reached 30% for seedlings less than 50 cm tall. Within 3 years, the fungus had disseminated throughout the island of Tahiti and had infected nearly all the miconia plants up to 1400 m in montane rainforests. It was also found on the neighbouring island of Moorea without any intentional release there. Leaf damage on miconia canopy trees increased from 4% to 34% with elevation in permanent plots set up between 600 and 1020 m. Our study showed that rainfall and temperature were two limiting environmental factors that affected fungal spread and disease development. Although this plant pathogenic agent is successfully established, has spread efficiently and has caused significant impacts on seedlings, additional biocontrol agents are still needed to fully control the massive invasion by miconia in the Society Islands.

***Keywords***: biological control, island, monitoring; rainforest.

## Introduction

'The greatest weakness of biological control has been the failure to quantify success adequately and to monitor programs effectively' (Myers and Bazely, 2003: 171).

Long-term monitoring of biological control agents in areas of introduction is essential to evaluate their effectiveness, i.e. their establishment (or acclimatization), their reproduction (or replication), their dissemination and their impacts on the target invasive alien species. Success of biocontrol programmes against weeds in agro-ecosystems or invasive plants in natural ecosystems is considered to be achieved if there is a notable reduction in population density through a decrease in the plant vigour, growth rate, ability to reproduce or to germinate (Briese, 2000; Myers and Bazely, 2003). It is also important to verify that the released natural enemy, although proven to be host-specific in laboratory conditions, does not affect other non-target species in natural conditions (Barton, 2004).

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This paper summarizes the results of monitoring a fungal pathogen introduced in a tropical island ecosystem to control a dominant invasive tree in native rainforests. We documented the establishment and dissemination of the plant pathogenic agent, quantified its direct impacts on the target species in the wild and verified the absence of damages on non-target plant species over a 6-year period. Factors suspected to have influenced the dynamics and impacts of the plant pathogen are discussed, and its general efficiency is assessed.

# Methods and materials

## Miconia, the 'green cancer' of Pacific islands native forests

Miconia, *Miconia calvescens* DC (Melastomataceae), represents one of the most dramatic and devastating cases of a documented plant invasion into island ecosystems. A small tree 4–12 m tall (up to 16 m in its native range), miconia is native to tropical rainforests of Central and South America where it is an understory species in dense forest and a colonizer of small forest gaps (Meyer, 1994). It was introduced to the tropical oceanic island of Tahiti (French Polynesia, South Pacific Ocean) in 1937 as a garden ornamental plant because of its large, striking leaves with purple undersides (under the horticultural name *Miconia magnifica* Triana). Rapid vegetative growth (up to 1.5 m per year), early sexual maturity (reached in four to five years), self-pollination and independence from specific pollinators, three flowering and fruiting peaks per year, prolific fruit and seed production (millions of tiny seeds per tree), active dispersal of the small fleshy berries by frugivorous native and alien birds over long-distances, high rate of seed germination (90% in 15–20 days in laboratory conditions) even under very low light conditions, large size and persistence of the soil seed bank (up to 10,000 seeds/m$^2$ and longevity more than 15 years) and shade-tolerance make this species a particularly aggressive colonizer in undisturbed native forests and a competitor with native and endemic insular species (Meyer, 1994, 1996, 1998). In less than 50 years, miconia has successfully invaded all the native mesic and wet forests of Tahiti (rainfall >2000 mm/year) from sea level to 1400 m elevation and covers approximately 70% of the island. Between 40 and 50 species of the 100 plants endemic to Tahiti are believed to be on the verge of extinction due to the invasion of miconia (Meyer and Florence, 1996). Miconia is also invasive in the rainforests of Hawaii (Medeiros *et al.*, 1997), New Caledonia and north Queensland in Australia, and remains a potential threat for many other wet tropical Pacific islands.

Because conventional manual and chemical control methods have shown their limits in heavily invaded islands such as Tahiti and Moorea (more than 80,000 and 3500 ha, respectively), where miconia is forming dense monospecific stands on steep, mountainous slopes, biological control is viewed as the only effective alternative.

## Cgm, a host-specific fungal plant pathogen

*Colletotrichum* (Order Melanconiales, Class Coelomycetes, Subdivision Deuteromycetinae) is one of the most important genera of plant pathogenic fungi worldwide, particularly in subtropical and tropical regions (Prusky *et al.*, 2000). There are several form species (or strains) of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. which are very specific to their plant hosts, including the well-known *C. gloeosporioides* forma specialis *aeschynomene*, which is used as a mycoherbicide registered as Collego® for control of the invasive legume, *Aeschynomene virginica* (L.) Britton, Sterns & Poggenb. (Fabaceae), in soybeans and rice in several Mississippi River delta states, and *C. gloeosporioides* (Penz) Sacc. f. sp. *jussiaeae* successfully used as a biological agent for control of winged water primrose, *Jussiaea decurrens* DC (Onagraceae) on rice fields of Arkansas (Templeton, 1982; TeBeest, 1991). In the Pacific Islands, *C. gloeosporioides* (Penz) Sacc. f. sp. *clidemiae*, found in Panama, was released in Hawaii in 1986 to control the invasive shrub, *Clidemia hirta* D. Don (Melastomataceae) (Trujillo et al., 1986; Trujillo, 2005).

*C. gloeosporioides* (Penz) Sacc. f. sp. *miconiae* Killgore & L. Sugiyama (hereafter, *Cgm*) was discovered in the State of Minas Gerais in Brazil in 1997 and isolated by Dr Robert Barreto (Universidad de Viçosa). It reproduces by asexual spores or conidia, 14.7–17.5 µm in length and 5.0–6.25 µm in width, which are produced in acervuli that arise on the abaxial surface leaf (Killgore *et al.*, 1999). Conidia of *Colletotrichum* fungi are produced under high moisture conditions and are disseminated by wind-driven rain. In the laboratory, test plants were inoculated using a spore concentration of $1 \times 10^5$ conidia millilitre in sterile water and incubated for 48 h in an enclosed chamber at 100% relative humidity (Killgore, 2002). The *Cgm* causes foliar anthracnose and necrosis, which lead to premature defoliation. When the pathogen is inoculated onto injured stems, cankers develop causing a dieback of the branch. Under aseptic laboratory conditions, the fungal pathogen attacked germinating miconia seeds and also killed emergent seedlings (Killgore, 2002). Mortality rate of very young seedlings (1 to 1.5 months old, less than 5 mm tall and with two leaves) in laboratory conditions (at room temperature between 24 and 30°C, hygrometry between 40 and 70%, 12 h light and 12 h darkness lightning regime) was 74% only 1 month after inoculation (Table 1).

Host-specificity tests were conducted at the quarantine facilities of the Hawaii Department of Agriculture in Honolulu following Wapshere's phylogenetic centrifugal method (Wapshere, 1974) on 28 plant spe-

**Table 1.** Mortality rate of young *Miconia calvescens* seedlings (1 to 1.5 months old) infected by *Colletotrichum gloeosporioides* f. sp. *miconiae* in laboratory conditions.

| Treatment | Inoculated (*N* = 163) | Control (*N* = 166) |
|---|---|---|
| Number of dead seedlings 1 week after inoculation date (%) | 99 (61%) | 1 (0.6%) |
| Number of dead seedlings 2 weeks after inoculation date (%) | 115 (71%) | 6 (4%) |
| Number of dead seedlings 4 weeks after inoculation date (%) | 120 (74%) | 6 (4%) |

cies in the botanical order *Myrtales* (species from the families Combretaceae, Lythraceae, Melastomataceae, Myrtaceae and Thymelaeaceae), including native and endemic Tahitian Melastomataceae (*Astronidium* spp. and *Melastoma denticulatum* Bonpl. ex Naudin). Repeated tests showed that the *Cgm* was highly specific to miconia (Killgore *et al.*, 1999). It was released in the Hawaiian Islands in June 1997 after approval by the US Department of Agriculture, the US Department of Interior and the Hawaii State Department of Agriculture and in Tahiti with the approval of the French Polynesian Government. The two release sites in Tahiti were the Taravao Plateau on the peninsula of Tahiti Iti in April 2000 and near Lake Vaihiria at the centre of Tahiti Nui in September 2002. Both sites are located at approximately 600 m elevation with an annual rainfall of 3300 and 7000 mm/year, respectively.

## Monitoring impact and dissemination

Monitoring was conducted in two permanent plots of approximately 100 m$^2$ set up in the two release sites in Tahiti for a 6-year period (2000–2006). A total of 110 seedlings and juvenile miconia plants between 20 cm and 1.5 m tall on Taravao and between 10 cm and 2.8 m tall on Vaihiria were tagged per plot. It was almost impossible to study smaller seedlings and larger plants for technical reasons (size of the tags and access to higher branches and leaves). Miconia plants were inoculated by spraying a solution of *Cgm* spores (concentration of $1 \times 10^8$ conidia millilitre in sterile water), which were mass-cultured in laboratory conditions (on 10% V8® juice agar and under constant fluorescent illumination at 20–24°C) at the Institut Louis Malardé in Tahiti from an inoculum sent by the Hawaii Department of Agriculture.

Every week for the first 3 months after the release, then every 6 months during the monitoring period, we recorded the number of dead plants per plot and the percentage of infected leaves (with one or more foliar lesions) for each plant; we counted the number of leaf spots on the most infected leaf for each plant and visually estimated the percentage of leaf damages (i.e. leaf area loss due to necrosis) on the most infected leaf for each plant (called ‘maximum damages’). A visual check for non-target damage was done at the same time, in and around the two permanent plots for disease symptoms. In particular, species belonging to the genus *Astronidium*, an endemic shrub of the same family as miconia, were examined.

A total of 20 transects, 10 m long, were set up in 2002 and 2003 on different parts of the island of Tahiti (leeward side, windward side, peninsula of Tahiti Iti) between 70 and 1100 m elevation to evaluate *Cgm* dispersal across the island. Observations on isolated plants were also made at higher elevation (up to 1400 m) and in other mountainous areas above 1000 m elevation in the centre of Tahiti during field surveys in 2003 (Fig. 1). Maximum leaf damages on mature, reproductive miconia trees (called ‘canopy leaves’) was measured in 2005 and 2006 on 28 cut trees (three to eight trees per site for a total of 1571 leaves) in eight other permanent plots located in different sites on the island of Tahiti between 600 and 1020 m elevation.

# Results

## Establishment and reproduction

Typical symptoms of *Cgm* infection appeared between 21 (Taravao) and 33 days (Vaihiria) after the initial inoculation. Subsequent re-infection by the fungus on miconia plants located outside the release plot (i.e. fungal reproduction and dispersal) varied between 3 (Vaihiria) and 18 months (Taravao) after inoculation. The difference in time to onset of re-infection between the two sites (located at the same elevation) may be explained by a much lower mean annual rainfall in Taravao (3300 mm/year) compared to Vaihiria (7000 mm/year) and by a serious drought period, which occurred in Tahiti when the pathogen was released on Taravao in 2000 (rainfall of 144 and 216 mm during the months of September and November 2000, respectively, vs a mean annual rainfall of 212 and 355 mm in September and November during the last 10 years. Data received from Météo-France in French Polynesia, personal communication).

## *Dissemination*

Within 3 years (2003), the fungus was detected 15 km from the release sites and spread throughout the island of Tahiti. It has infected almost all the miconia trees, juvenile plants and seedlings between sea level and 1400 m elevation. Miconia plants found on the neighbouring island of Moorea (located approximately

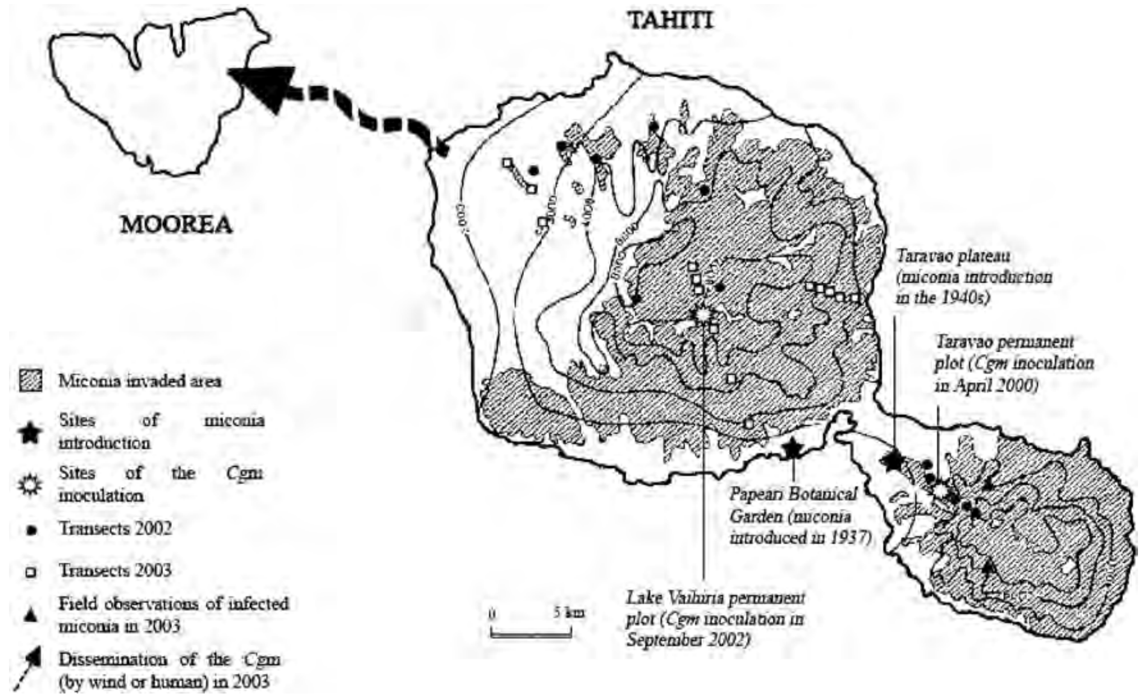


**Figure 1.** Map of *Miconia calvescens* invasion in Tahiti and the evolution of *Colletotrichum gloeosporioides* f. sp. *miconiae* dissemination from the two release sites.

20 km from Tahiti) were also infected by *Cgm* in 2003 without being intentionally introduced (Fig. 1). Spores may have been carried there by the wind or on contaminated clothing or equipment.

## Impacts

Three months after inoculation, 100% of the tagged plants and between 90% and 99% of their leaves were infected by *Cgm* (i.e. presence of leaf spots) in the two permanent plots (Figs. 2 and 3). The mean number of leaf spots on the most infected leaf increased from 10 (1 month after the inoculation) to 90 (6 years after the inoculation) in Taravao, and from 10 (1 month after the inoculation) to 55 (4 years after the inoculation) in Vaihiria. In 2006, the mean mortality rate was approximately 15% for the monitored plants (14.1% in Taravao and 16.7% in Vaihiria), reaching approximately

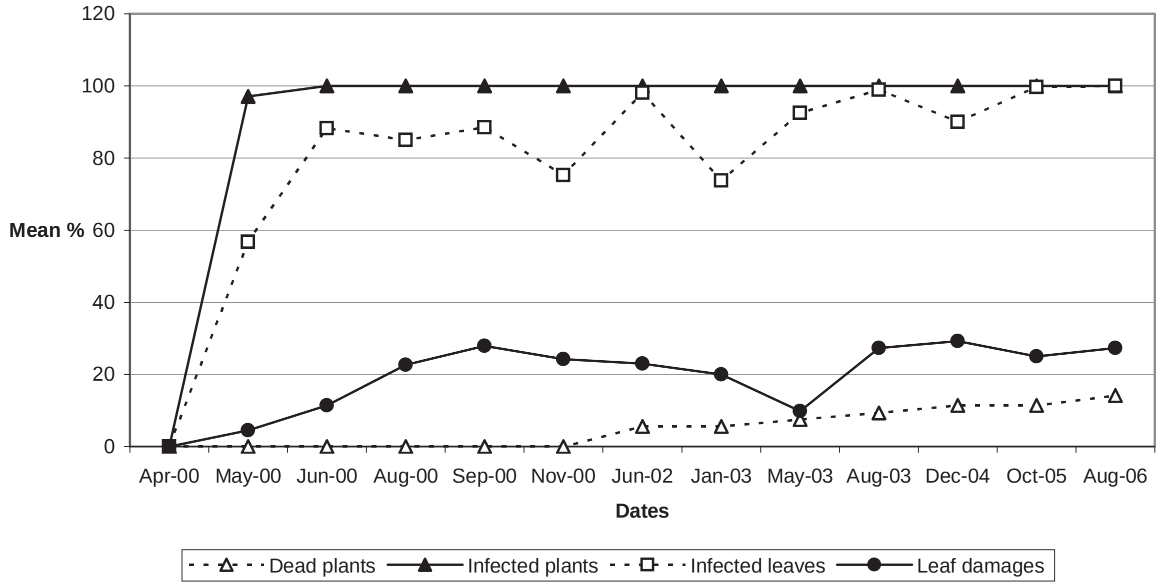


**Figure 2.** Evolution of *Colletotrichum gloeosporioides* f. sp. *miconiae* impacts on monitored *Miconia calvescens* plants in the Taravao release site (2000).

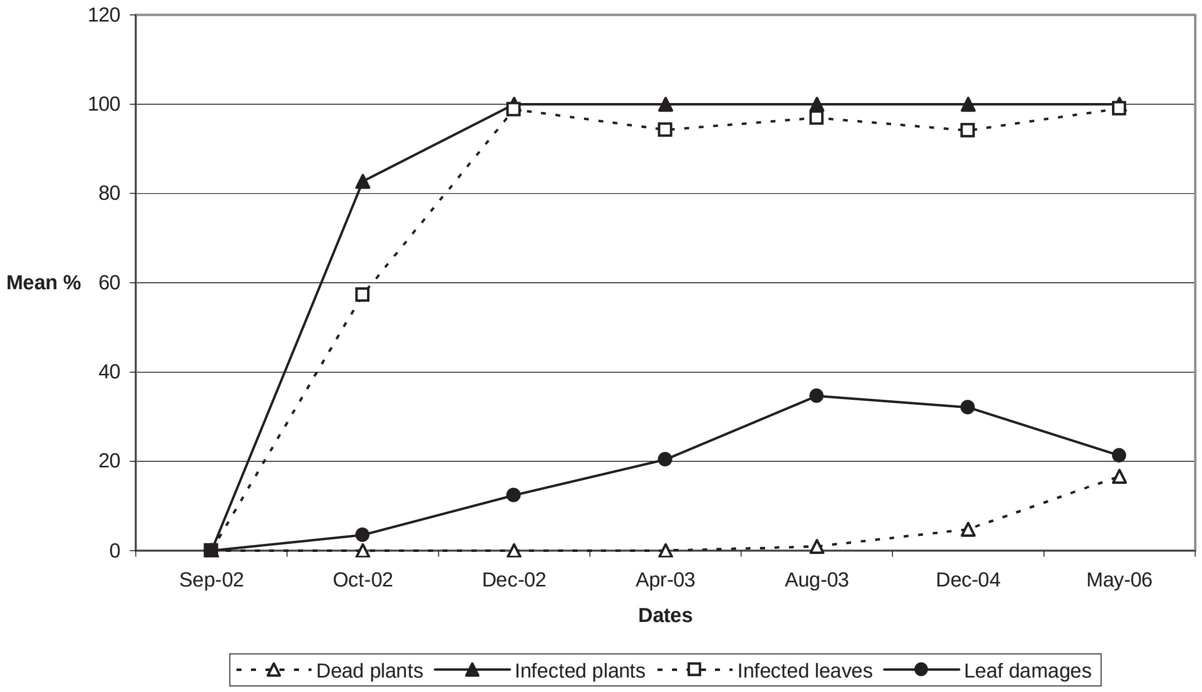


**Figure 3.** Evolution of *Colletotrichum gloeosporioides* f. sp. *miconiae* impacts on monitored *Miconia calvescens* plants in the Vaihiria release site (2002).

30% for small seedling less than 50 cm tall (28.6% in Taravao and 32.3% in Vaihiria). Multiple damages on the surviving tagged miconia plants, including rotting stems and deformed leaves, ranged between 14% (Taravao) and 47% (Vaihiria). Mean maximum leaf damages on the tagged miconia plants in the two monitored sites was approximately 25% (27.4% in Taravao and 21.3% in Vaihiria), and maximum damages on miconia canopy leaves in eight other permanent study sites (100 $m^2$ quadrats) varied between 4% (at 600 m elevation) and 34% (at 970 m elevation; Fig. 4). None of the non-target alien and native plants, located within or outside the permanent plots, displayed any symptoms of *Cgm* infection after 6 years of monitoring.

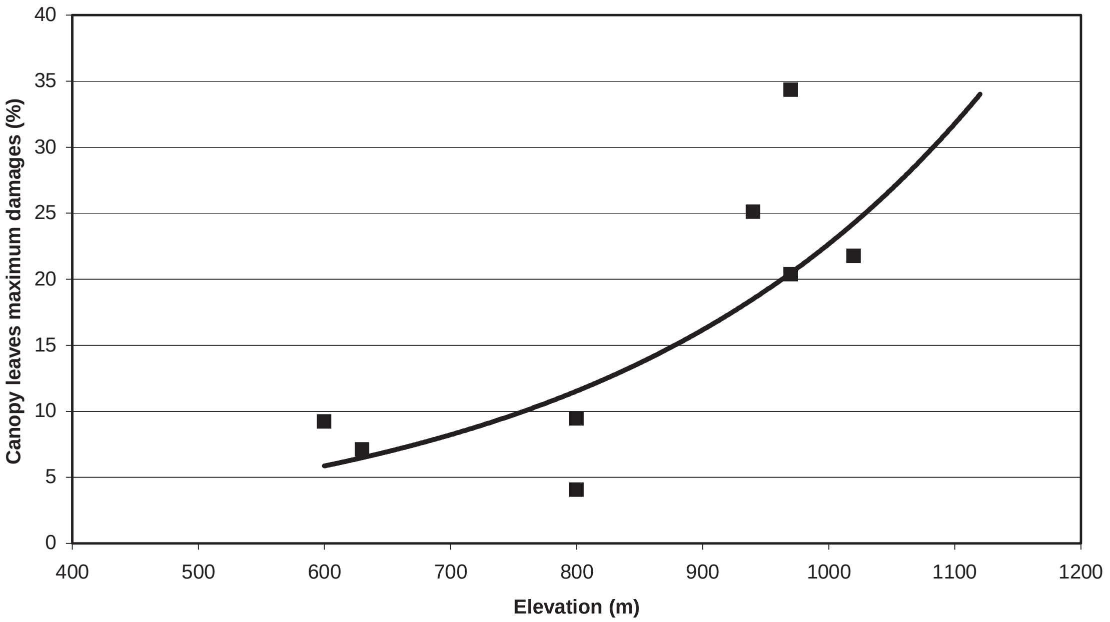


**Figure 4.** Mean percentage of *Miconia calvescens* canopy leaves maximum damages caused by *Colletotrichum gloeosporioides* f. sp. *miconiae* according to elevation in eight different plots set up in Tahiti in 2005–2006 ($N$ = 1571 leaves).

## Discussion

Only a very few biocontrol programmes using plant pathogens with the goal of preserving tropical island native forest ecosystems are documented (Gardner, 1992; Smith *et al.*, 2002; Trujillo, 2005) compared to their common use in agriculture ecosystems (see, e.g. Templeton, 1982). In natural areas, the need to carefully monitor and evaluate agent establishment, dissemination and impact is particularly important, so that the degree of success can be quantified or reasons for failure can be understood (Briese, 2000).

The *Cgm*, a fungal pathogen proven to be highly-specific to miconia in laboratory conditions, was successfully introduced to the tropical oceanic island of Tahiti in 2000 (Meyer and Killgore, 2000). Our results show that in 3 years, it has established, reproduced and spread throughout the island of Tahiti and even to the neighbouring island of Moorea, located approximately 20 km away, without any intentional release there. The *Cgm* has succeeded in infecting almost all the miconia plants on both islands without causing any apparent harm to non-target plant species. It was capable of distributing itself by natural means, particularly at high elevation in montane rainforests or cloudforests (up to 1400 m elevation), and to infect both dense monospecific stands and isolated miconia plants. Several biocontrol programmes elsewhere in the world have been considered unsuccessful (or only partially successful) because of the large habitat range of the target species but the narrower ecological range of their natural enemies (e.g. *Lantana camara* L. in Hawaii; Broughton, 2000).

Leaf damage caused by *Cgm* is more severe in high-elevation areas of Tahiti (Moorea and Raiatea, unpublished observation) where cooler and wetter conditions prevail, suggesting that temperature and moisture (as humidity or free water) are important factors for disease development. The reproduction and dissemination of the pathogen was delayed at the Taravao site due to a drought period, which occurred when the pathogen was released in 2000. The same pattern was observed in Hawaii after the release of the *Cgm* in 1997. The importance of air temperature was demonstrated for other *C. gloeosporioides* with an optimum temperature for many form species at about 28°C. Disease development was severely limited at 36°C (TeBeest, 1991). Defoliation of *C. hirta* caused by *C. gloeosporioides* f. sp. *clidemiae* over contiguous areas only occurs when weather conditions are favourable, i.e. cool (16–24°C), windy and rainy (Conant, 2002; Trujillo, 2005; R. Hauff, personal communication).

Mortality rate was high for very young miconia seedlings (74% 1 month after inoculation) in laboratory conditions but was relatively low and slow for larger miconia plants in the field (15% for plants between 10 cm and 2.80 m tall, up to 30% for seedlings less than 50 cm tall, 4 to 6 years after the release). Although the plant pathogen may slow the growth of established miconia plants (between 17% and 35 % of the surviving miconia plants showed multiple damages, especially rotten stems and curved leaves) and cause the dieback of young seedlings, it alone will not control the massive invasion of miconia. Partial defoliation of reproductive canopy trees (up to 35%) favoured the recruitment of native plants, including rare threatened endemic plants, by enhancing the light availability in the understory (Meyer *et al.*, 2007).

The *Cgm* was released on the island of Raiatea (Society Islands) in 2004, where manual and chemical control operations have been conducted since 1992 on a 450-ha infested area, and in Nuku Hiva (Marquesas Islands) in 2007, where small miconia populations (<1 ha) were recently discovered. On these islands, manual and chemical control will be necessary to achieve complete eradication, but the fungal pathogen is expected to reduce the number of miconia plants, especially those at the seedling stage. Additional biological control agents are still much needed to fully control the massive invasion by miconia in the Society Islands and the Hawaiian Islands.

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# One agent is usually sufficient for successful biological control of weeds

**J.H. Myers**

*Summary*

A review of the recent literature reveals ten new cases of successful biological control of weeds. In each of these a single species of biological control agent has apparently caused the decline in the density and biomass of the target weed. Each of these agents also dramatically damages the plants and reduces their survival. While it remains difficult to predict which species of insect herbivore or plant pathogen will be successful, history suggests that those that cause serious damage to the plants are most likely to reduce target weed biomass and density. Clearly single agents can be successful.

***Keywords***: biological control efficacy, natural enemies, invasive plants.

## Introduction

Recent, highly publicized examples of non-target impacts in weed biological control programmes (Louda *et al.*, 2003) have resulted in more stringent regulations and public apprehension about the introduction of new, exotic biological control agents. In addition, interest in the conservation of natural biodiversity calls attention to the potential impacts of more introductions of exotic species such as those occurring in weed biological control programmes. Given these concerns, in the future, it will be important for the practice of biological control to be conservative in terms of the number of agents introduced and to be efficient in regard to the degree of control achieved with each new species introduced.

Although it is a commonly held view that biological control success requires the introduction of several species of agents attacking different parts of a target weed, we have shown in the past that a majority of weed biological control successes has been attributed to a single species of agent (Denoth *et al.*, 2002). To determine if the pattern in more recent weed biological control successes is in accordance with the earlier analysis, I have reviewed the recent literature for successful weed control projects to determine if single or multiple agents were required to achieve biological control success.

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## Methods

The definition of success that I have used in this evaluation is that the population density of the target weed was greatly reduced by the activity of the introduced agent. Even if the scale at which the agent had established was small and geographically limited, I considered this to be successful control if plant density at sites with established agents declined significantly as compared to control sites lacking the agent. Other definitions of success might include factors such as the geographical extent of the impact of the control agent or other biological parameters such as the reduction of seed production (review in Myers and Bazely, 2003).

The recent literature was searched using Biosis and Science Citation Index and the key words ‘biological control success’. I also reviewed the journal *Biological Control* from 2003 to the present for examples of successful control programmes.

## Results

Nine cases were found of biological control of weeds programmes recorded in the recent literature, and we have been studying an additional example that is successful (Table 1). In all these cases, success was attributed to a single agent, and in no recent cases were multiple agents reported to be necessary or involved in success. These examples are briefly described below.

**Table 1.** Summary of recently reported successful biological control of weed programs. Est. indicates the number of agents that are established in the program. Successful agents are those to which success has been attributed in the literature.

| Weed | Country | Est. | Successful agents | Reference |
|---|---|---|---|---|
| *Mimosa pigra* | Australia | 8 | *Carmenta mimosa* | Paynter (2005) |
| *Asparagus asparagoides* | Australia | 3 | *Puccinia myrsiphylii* | Morin and Edwards (2006) |
| *Linaria dalmatica* | Canada–BC, USA | 1 | *Mecinus janthinus* | De Clerck-Floate and Harris (2002), Nowerski (2004), Hansen (2004) |
| *Azolla filiculoides* | South Africa | 1 | *Stenopelmus rufinasus* | McConnachie *et al.* (2004) |
| *Ageratina riparia* | New Zealand | 2 | *Entyloma ageratinae* | Barton *et al.* (2007) |
| | Hawaii | 3 | | Trujillo (2005) |
| *Passiflora tarminiana* | Hawaii | 1 | *Septoria passiflorae* | Trujillo (2005) |
| *Cynoglossum officinale* | Canada | 1 | *Mogluones cruciger* | De Clerck-Floate and Schwarzländer (2002) |
| *Lythrum salicaria* | Canada | 1 | *Galerucella calmariensis* | Lindgren *et al.* (2002), Denoth and Myers (2005) |
| *Centaurea stroebe* | Montana | 12 | *Cyphocleonus achates* | Story *et al.* (2006) |
| *Centaurea diffusa* | British Columbia, Colorado, Montana | 12 | *Larinus minutus* | Myers (unpublished), Seastedt *et al.* (2003), Smith (2004) |

## Mimosa, *Mimosa pigra*, L.

*M. pigra* is a woody legume that creates impenetrable stands in the Northern Territory of Australia (Paynter, 2005). Six biological control agents were introduced and established, but of these, only the twig boring moth, *Carmenta mimosa* Echlin and Passoa, has been found to reduce the density of *M. pigra*. Attack by *C. mimosa* can cause severe defoliation and kill twigs and branches, and this can reduce seed production by 90%. Reduced seed banks are associated with increased under storey from competing plants, and this further hinders seedling success. Higher densities of understorey plants also increase fuel loads and the susceptibility of mimosa to fire. Mimosa populations did not expand in any of the nine sites in which *C. mimosa* was established and three of the sites contracted in size. In contrast, mimosa populations expanded in four of eight areas that lacked *C. mimosa*. From this study (Paynter, 2005), it appears that *C. mimosa* has the potential to be a successful biological control agent.

## Bridal creeper, *Asparagus asparagoides*, L. Druce,

Bridal creeper, *A. asparagoides,* is a South African vine that has invaded natural areas with Mediterranean climates in Australia. Initially, it was considered that multiple agents would be required for biological control success, and three agents were introduced: a rust, *Puccinia myrsiphylli* (Thüm), a leafhopper in the genus *Zygina* and a beetle in the genus *Crioceris* (Morin and Edwards, 2006). Of these, the rust has become widely established in coastal areas and has significantly reduced bridal creeper populations (Morin *et al.*, 2006). Although defoliation by the leafhopper also appears to have the potential to reduce the population density of bridal creeper, an egg parasite has reduced its success and populations are unstable. It may have a greater impact in drier, interior sites where the conditions may be less favourable for the fungus (Morin and Edwards, 2006). Presently, it appears that the fungus is able to achieve successful biological control on its own in coastal areas of Australia.

## Dalmatian toadflax, *Linaria dalmatica*, L. (Mill.)

Dalmatian toadflax, *Linaria dalmatica*, infests large areas of rangeland in western North America. The stem-boring weevil, *Mecinus janthinus*,Germar, has been highly successful in reducing densities of Dalmatian toadflax, particularly in British Columbia, Canada (De Clerk-Floate and Harris, 2002; Hansen, 2004; Nowerski, 2004; McClay and Hughes, 2007). In some areas such as Alberta, Canada, the climate is less favourable for the weevils, and they do not have sufficient time to develop to the over wintering adult stage (McClay and Hughes, 2007). Thus, *M. janthinus* is an example of a species that is a successful biological control agent on its own in some regions, but it is not adapted to all of the areas invaded by the target weed.

## Red floating fern, *Azolla filiculoides*, Lam.

Red floating fern, *A. filiculoides*, is native to South America but became a serious aquatic weed in South Africa. The frond-eating weevil, *Stenopelmus rufinasus*, Gyllenhall was introduced from Florida, USA,

to South Africa in 1997 and caused extinction of the water fern at 81% of the original 112 release sites usually within 7 months of its release (McConnachie *et al.*, 2004). This programme with a single species of agent is considered to be highly successful.

## Mistflower, *Ageratina riparia* (Regel) R.M. King & H. Rob

Mistflower, *A. riparia,* is an ornamental that is native to Central America. It has been spread to many tropical areas where it reaches dense stands in warm, moist habitats along forest edges, wetlands and poorly managed pastures. In HawaiI, four species of biological control agent were introduced, and success was attributed to all of them, although no quantitative data were collected (Trujillo, 1985). Two species of biological control agents were introduced to New Zealand: the white fungus, *Entyloma ageratinae* Barreto and Evans, in 1998, and a gall fly, *Procecidochares alani*, Steyskal, in 2001. The fungus spread rapidly, and within several years, mistflower cover declined from 81% to 1.5% at some heavily infested sites, apparently before the gall flies were widely established (Barton *et al.,* 2007). The success of *E. ageratinae* in reducing mistflower suggests that this species may be sufficient for successful biological control, and the contribution of other agents in Hawaii may have been overestimated.

## Banana poka, *Passiflora tarminiana*, Copens and Barney

Banana poka, *P. tarminiana*, is native to the high Andes and became a major weed in high elevations of Hawaii where it infested more that 50,000 ha (Trujillo, 2005). In 1993, a fungus, *Septoria passiflorae* Syd., was introduced to Hawaii from Columbia for host-range testing under quarantine. After it was shown to be specific, *S. passiflorae* was sprayed on banana poka in the Hilo Forest Reserve. Densities of the weed were reduced by 95% within 4 years in many areas but not in regions in which the fungus was killed by acid rain (Trujillo, 2005). This plant pathogen has preserved endangered species and allowed the regeneration of koa forests at high elevations on the islands of Kauai, Maui and Hawaii.

## Houndstongue, *Cynoglossum officinale* L.

Houndstongue, *C. officinale,* is a native of Europe and Asia Minor and a serious rangeland weed in British Columbia, Canada. The seeds of this plant attach to the faces and hides of cattle and the foliage is very toxic to large mammals. The root-boring weevil, *Mogluones cruciger* Herbst., was introduced in 1997 and became effectively established at many sites where it may attack more than 90% of flowering plants and kill over half the rosettes it attacks in the autumn. *M. cruciger* has reduced the density of houndstongue at most of the release sites (De Clerck-Floate and Schwarzländer, 2002; De Clerck-Floate *et al.*, 2005) and thus is considered to be a successful biological control agent.

## Purple loosestrife, *Lythrum salicaria* L.

Purple loosestrife, *L. salicaria*, is a European plant that has become widely spread in wetland areas across southern Canada and the northern USA. Two species of leaf-feeding beetles have been introduced widely as biological control agents, *Galarucella calmariensis* and *Galarucella pusilla* Dust., and in some locations, the root-boring beetle, *Hylobius transversovittatus* Goeze, has also been introduced. In southern British Columbia, the species composition of releases is not certain but is likely to have been dominated by *G. calmariensis* (R. DeClerk-Floate, personal communication). This has effectively reduced loosestrife at several sites that were monitored (Denoth and Myers, 2005). Similarly, in Manitoba, *G. calmariensis* reduced loosestrife populations at several locations (Lindgren, 2000). In Michigan, both species of beetles were introduced, but only *G. calmariensis* became established and here too the species effectively reduced loosestrife density at many locations (Landis *et al.*, 2003). Both *Galarucella* spp. were established in western Oregon and were considered to be ecological equivalents. They contributed to a dramatic decline in loosestrife plant size and density over 3 years (Schooler, 1998). In New York, *G. pusilla* was the dominant species at many locations, but neither species together or alone had an apparent impact on loosestrife density (Grevstad, 2005). After 5 years, *G. calmariensis* was spreading and increasing in density compared to *G. pusilla*. In another study, in New York, the two species of leaf-feeding beetles were introduced, and after 5 years, a significant decline in loosestrife densities occurred (Albright *et al.*, 2004). The species composition of the two beetle populations was not monitored over time in this study so it is not clear if both species were involved in the decline of host plant density. Comparisons of the impacts of *H. transversovittatus* and *G. calmariensis* showed that the leaf-feeding beetle had the greatest impact on loosestrife reproductive effort and above-ground biomass, and this damage allowed improved growth of other plants in plots (Hunt-Joshi *et al.*, 2004). In conclusion, reduction of purple loosestrife density has been achieved in programs involving only *G. calmariensis,* and this shows that a single agent can be successful. The failure of Grevstad (2005) to find a reduction in loosestrife density in programs that initially involved both leaf-feeding beetle species may suggest that there can be a negative interaction of the species or that the environment in New York is less conducive to control by these leaf-feeding beetles. The two species were successful in combination in western Oregon however.

## Spotted knapweed, *Centaurea stoebe Linnaeus micranthos* (S. G. Gmelin ex Gugler) Hayek

Spotted knapweed, *C. stoebe micranthos*, is a serious rangeland weed in western North America where it infests more than 3 million ha. Twelve Eurasian insect species have been introduced as potential biological control agents. Seven of these species are considered to have an impact through the reduction in seed production. This has not, however, been translated into successful reduction of plant density. One species, the root-boring beetle *Cyphocleonus achates* Fahraeus can kill spotted knapweed plants and caused a significant reduction in plant density, 99% and 77% over 11 years, at two sites where it reached high densities (Story *et al.*, 2006). This weevil does not fly, so its spread is slow, but nevertheless, the population did expand on average by 99 m per year. Thus, this species is capable of at least local control of spotted knapweed.

## Diffuse knapweed, *Centaurea diffusa* Lamarck

Diffuse knapweed, *C. diffusa*, like spotted knapweed, is a rangeland weed in western North America. It infests drier areas at lower elevations than spotted knapweed and shares some of the introduced biological control agents with spotted knapweed (Bourchier *et al.*, 2002). Twelve species of exotic, potential control agents have been introduced for this species as well, and the most widely established agents include two gall flies, *Urophora affinis* Frauenfeld and *U. quadrifasciata*, the root-boring beetle, *Sphenoptera jugoslavica* Obenberger, and most recently the weevil, *Larinus minutus* Gyllenhal. Since the establishment and spread of *L. minutus* in the late 1990s, densities of diffuse knapweed have declined in many sites in British Columbia, Canada and Colorado and Montana in the United States (Seastedt *et al.*, 2003; Smith, 2004; J. Myers, unpublished data). While the other established biological control agents reduce seed production of diffuse knapweed, *L. minutus* reduces both seed production and plant survival. Damage to flowering plants can be severe particularly in dry summers. The impact of *L. minutus* on diffuse knapweed in British Columbia was demonstrated after a fire removed both knapweed and biological control agents from a site. The seed bank of knapweed was sufficient for a resurgence of dense plant populations, and over 3 years, *L. minutus* returned to once again suppress the knapweed (J. Myers, unpublished data). These observations suggest that *L. minutus* is an effective control agent and sufficient to reduce the density of diffuse knapweed.

# Discussion

These examples of recent biological control successes can be incorporated with the data previously summarized by Denoth *et al.* (2002) (Fig. 1). In that review, based on Julien and Griffiths (1998) catalogue of biological control agents and weeds, diffuse knapweed

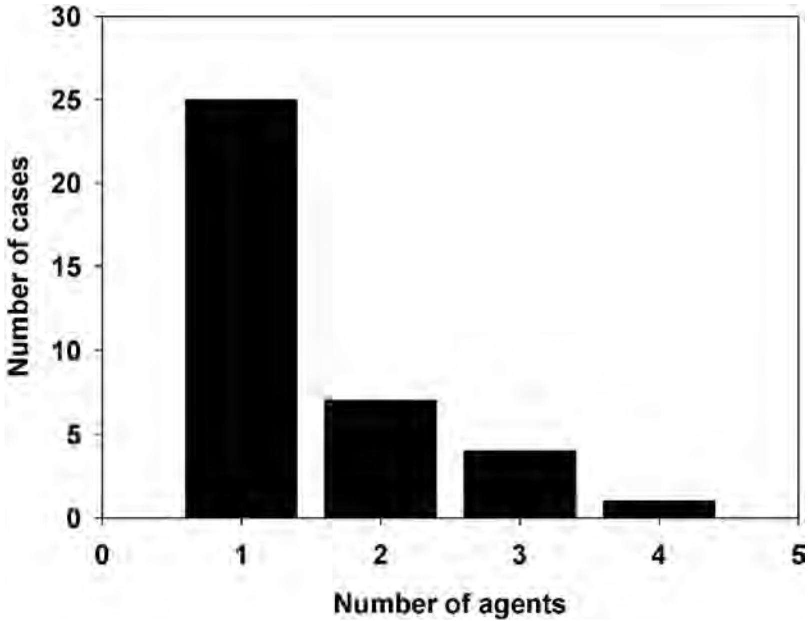


**Figure 1.** Number of agents considered to have contributed to the successful biological control of weeds. In this figure, ten new successful projects are added to those summarized by Denoth *et al*. (2002), and instead of attributing success to the eight agents established in biological control of diffuse knapweed programs, with the success of *Larinus minutus*, this project is moved to the category in which one agent was successful.

control was attributed to the combined impact of eight agent species. This is curious because diffuse knapweed densities in British Columbia, upon which this value was based, had not declined before 1998. Therefore, I suggest that this be changed, as the decline of diffuse knapweed occurred after the establishment of *L. minutus* that is likely the agent most responsible for the successful control of diffuse knapweed, and I have incorporated this change in Fig. 1.

One situation in which multiple species of agents may be necessary is when the target weed has a greater environmental range than the agent. For example, the failure of *M. janthinus* to survive in Alberta, Canada suggests that another agent or strain of control agent may be necessary in the toadflax control programme (McClay and Hughes, 2007).

In this review, I only considered published cases of biological control success. Less successful programmes are unlikely to be described in publications, and thus, we cannot evaluate the impacts of the biological control agents in these. Cases of modest control by several agents may therefore be overlooked and understudied (M. Julien, personal communication). An exception is a recent report on the impact of the mite *Tetranychus lintearius* Dufor, on gorse, *Ulex europaeus* L. (Davies *et al.*, 2007). This agent was originally recommended for use in gorse control by Zwölfer (1963) who observed dead plants in their native habitat that he thought had been killed by the mites. In the study of Davies *et al.* (2007) and others reviewed there, the mites have been found to reduce the growth and sometimes flower production of gorse. Damage from *T. lintearius* and five other species of biological control agents has been insufficient, however, to successfully control gorse measured as a reduction in the density of plants (Hill *et al.*, 2000).

Although a majority of successful weed programmes can be attributed to a single agent, this observation does not help to identify the characteristics that will allow agents to be successful before their release. One factor that does stand out however is the ability of the agent to kill the target weed. It appears that accumulated impact of several types of feeding damage from different agents is not necessary for successful control. To reduce the number of releases of exotic species for biological control, the paradigm should change from the assumption that multiple agents are required to a greater focus on identifying potential biological control agents that can kill host plants. Introducing fewer agents per programme would make biological control of weeds more efficient, less costly and more environmentally benign. More focus on the potential efficacy of biological agents could be cost effective (McClay and Balciunas, 2005).

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# Evaluating implementation success for seven seed head insects on *Centaurea solstitialis* in California, USA

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*Summary*

A stage-based invasion model proposed by Colautti and MacIsaac (2004) is used to evaluate the success of implementation efforts for seven seed head insects introduced on *Centaurea solstitialis* L. (Asteraceae) in California, USA. Six insects were introduced intentionally; one was introduced unintentionally. Establishment of initial foreign material (stage III invasion) at field nursery sites was very successful (86%) with six of seven species establishing (five of six intentional, one of one unintentional). For the intentional species, initial releases occurred at multiple locations, and establishment success among locations was 100% for four of the five species that established. Statewide distribution of seed head insects introduced intentionally (stage IV invasion) was performed through the network of County Agricultural Commissioners. Training workshops provided knowledge and insects to county biologists for release in their county. Hundreds of releases occurred in this way, and follow-up monitoring estimated establishment success above 87% for four of six species. Regional spread and population increase to high density (stage V invasion) was examined in an independent statewide survey of *C. solstitialis* seed heads in 2001 and 2002. Results showed that only one of the intentional species, *Eustenopus villosus* (Boheman) and the unintentional species, *Chaetorellia succinea* (Costa), appear to have achieved high densities over large areas. The fly, *C. succinea*, appears to be the most successful seed head insect, while *E. villosus* was second in abundance statewide. The other seed head insects were recovered in low numbers statewide and appear to contribute little to the herbivore pressure on this weed.

***Keywords***: invasion, stage-based implementation model.

## Introduction

Development of a biological control organism may be grouped into pre-release and post-release research efforts. Pre-release efforts include the foreign exploration and host-specificity testing of candidate natural enemies, among other efforts (Harley and Forno, 1992; Briese, 2000). Post-release efforts begin with the approval to release the control organism from quarantine and consist of release and establishment efforts, field impact studies and non-target surveys (Hansen, 2004). The multi-year release and establishment effort is the ‘implementation phase’ of a new biological control organism and is commonly carried out by state and local government entities. This phase occurs in several steps: initial establishment of foreign material in field nursery sites; distribution of domestic material from nursery sites to satellite locations throughout the region; and eventual spread of the natural enemy away from release sites into the surrounding infestations of the target weed. The goal is to obtain self-sustaining populations of the biological control agent throughout the area infested by its host plant.

Several researchers have pointed out the similarity between the intentional introduction of a biological control organism and the invasion of an unintentionally introduced exotic organism (Crawley, 1989; Simberloff, 1989; Grevstad, 1999; McEvoy *et al.*, 2000). Colautti and MacIsaac (2004) and Colautti (2005)

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proposed a stage-based description of the invasion process over large geographic areas for invasive species. Here, we modify their model to describe the stages of the implementation phase of a biological control organism and use this model to evaluate the success of implementation efforts for a guild of exotic seed head insects introduced against *Centaurea solstitialis* L. (Asteraceae), commonly named yellow starthistle, in California (USA).

# Methods

## The invasion model

Colautti and MacIsaac (2004) and Colautti (2005) proposed a model of six stages to describe the invasion of an exotic organism into a new region. Stage 0 is the organism in its area of origin; in stage I, the organism is taken up by a transport vector (ship ballast, contaminant in a seed shipment, etc.); in stage II, the organism is introduced into the new region; in stage III, the organism establishes a small, incipient population; in stage IV, the organism spreads throughout the new region; and in stage V, the organism has spread over a large region and generally occurs in high density. Stage IV can be achieved through two processes: formation of several small satellite populations away from the initial infestation (stage IVa) or simple expansion of the initial established population (stage IVb). Both stages IVa and IVb expand to stage V.

The development and utilization of a biological control organism may be described in a similar way. Stage 0 is the natural enemy in its host's area of origin; in stage I, a sample of the natural enemy population is collected and held in quarantine for host-specificity testing; in stage II, a sample of the material in quarantine is released on the target weed in the new region; in stage III, the organism has established small populations in field nursery sites; in stage IV, biological control workers collect the organism from the initial nursery sites and distribute it throughout the region infested by the target weed. The goal is stage V, where the biological control organism forms self-sustaining populations of high density throughout the regions infested by the target weed. In this model, the implementation phase of a biological control organism begins with its approval to release (stage II) and ends with the biological control organism occurring in high density throughout the area infested by the target weed (stage V).

## Biology of yellow starthistle in California

Yellow starthistle is an invasive exotic weed from the Mediterranean region of Europe and was likely introduced into California as a contaminant in shipments of alfalfa seed (DiTomaso and Gerlach, 2000). It was first recorded near the San Francisco Bay in 1859 and now infests over 5.7 million ha in California alone (Pitcairn *et al.*, 2006). Yellow starthistle is a winter annual that invades rangelands, orchards, vineyards, pastures, parks and natural areas. It is favored by soil disturbance but can invade areas that have not been disturbed by humans or livestock. Germination begins with the onset of the winter rains; flowering begins in early summer and continues into the fall. Individual plants may produce from one to 100 capitula (hereafter, seed heads); infested areas commonly produce 650 to 700 seed heads per square metre, but densities as high as 3000 seed heads per square metre have been reported (DiTomaso *et al.*, 2003). In uninfested heads, approximately 30 to 40 achenes (hereafter, seeds) per head are produced. Yields of 50 million seeds/ha are common (M. J. Pitcairn, unpublished data), but estimates of 120 to 500 million seeds/ha have been recorded in heavily infested areas (Maddox, 1981; DiTomaso and Gerlach, 2000).

Yellow starthistle is the target of an ongoing biological control effort in the western United States. Research efforts resulted in the release approval of six exotic insects that oviposit in the immature seed heads, and their larvae feed on developing seeds. The first insect released was the gall fly, *Urophora jaculata* (Rondani) (*Dipt.*: *Tephritidae*), in 1969, but it failed to establish (Turner *et al.*, 1994). Five other insects were released from 1984 through 1992, and all have established: *Bangasternus orientalis* (Capiomont) (Col.: Curculionidae), *Eustenopus villosus* (Boheman) (Col.: Curculionidae), *Urophora sirunaseva* (Hering) (Dipt.: Tephritidae), *Chaetorellia australis* (Hering) (Dipt.: Tephritidae) and *Larinus curtus* Hochhut (Col.: Curculionidae) (Turner *et al.*, 1994). In California, three species, *B. orientalis*, *E. villosus* and *U. sirunaseva*, are now widespread. The two other insects, *C. australis* and *L. curtus*, occur in low numbers at a limited number of locations. A seventh insect, *Chaetorellia succinea* (Costa) (Dipt.: Tephritidae), was accidentally introduced into southern Oregon in 1991 and is also widely established throughout California (Balciunas and Villegas, 1999).

## Evaluation of the implementation stages

**Stage III - releases of foreign material:** The six insect species intentionally released as biological control organisms were collected from their area of origin (Greece, Italy and Turkey) as larvae in seed heads, sent to the United States Department of Agriculture, Agricultural Research Service quarantine facility in Albany, CA, USA and held for emergence in sleeve cages (Turner *et al.*, 1996). Emerged adults were collected, identified and a subsample killed and examined for internal parasites and diseases. When ready, the remaining adults were released into field nursery sites (Turner *et al.*, 1996). Most nursery sites were monitored annually for recovery of the insect and, when population levels were considered

high enough, the sites were used for training and a source of adults for distribution in stage IV.

**Stage IV - releases of domestic material:** Stage IV involves the transfer of knowledge and biological control organisms from state control to local control. The State of California is divided into 58 counties for the purposes of supporting local governmental activities. Each county supports an Agricultural Commissioner who maintains a small staff of biologists that assist with issues concerning local agriculture. The California Department of Food and Agriculture performs workshops and training sessions for the county biologists on the biology of biological control organisms and the damage they cause to the target weed (Villegas, 1998). Training occurs through oral presentations at field nursery sites and through active participation of the attendees in the collection, counting and packaging of biological control organisms. Afterwards, workshop attendees return to their counties and release the organisms at new field sites. This network of trained county biologists has been very effective in the distribution of biological control organisms throughout California.

A sample of stage IV release sites (*n* = 60–120) were monitored 3 to 4 years following their initial releases. The sites were chosen to represent the different climatic regions where yellow starthistle grows. Plants were swept with a sweep net at late bud stage or early flowering to collect any active adult seed head insects, and any evidence of oviposition and feeding damage was recorded.

**Stage V - local spread and population increase:** It was expected that each of the seed head insects would increase their densities locally and spread throughout the populations of yellow starthistle located nearby. The regional spread of the seed head insects away from their release sites was evaluated by a survey performed in 2001 and 2002 where plant samples were collected along roads throughout areas infested with yellow starthistle. Samples occurred approximately every 16 km. Over 100 seed heads from at least ten plants were collected, and the date and latitude and longitude coordinates for each sample location were recorded. All seed heads were returned to the laboratory, and a minimum of 100 heads from each sample was dissected, and the presence of insects was recorded by species. Each biological control organism produces a characteristic type of feeding damage that is easily recognized upon dissection of the head. The exception was the damage caused by the two species of *Chaetorellia*. A second survey where adult flies were reared from seed heads showed that *C. australis* was infrequently recovered, and its infestation rate was very low (<5%). Thus, for the analysis reported here, we consider all heads damaged by *Chaetorellia* spp. to be infested with *C. succinea*.

Typically, immature seed head production begins in May with peak production in early July (unpublished data). Seed heads accumulate on plants and provide a record of the cumulative attack by insects throughout the season. Survey collections began after peak seed head production (late July). A total of 421 samples were collected during the two summers of this study. A data file of insect abundance based on the proportion of seed heads infested was plotted using a geographic information system (GIS), and values between points were interpolated using an inverse-distance weighting (IDW) algorithm that covered the entire state of California.

# Results

## Stage III – releases of foreign material

Five of the six seed head insects released as approved biological control organisms established (Table 1). For each species, releases of foreign material occurred in three to seven locations within California (additional releases occurred in Oregon, Washington and Idaho; Turner *et al.*, 1994). Establishment rates among locations were 100% except for *U. jaculata*, which failed to establish, and for *C. australis*, which failed to establish at all five release sites in California. An overall establishment rate of 27% for *C. australis* is estimated from its rate of establishment among all locations in the other western states (Turner *et al.*, 1996).

The unintentional release of *C. succinea* occurred when foreign material from quarantine consisting of a mixture of *C. succinea* and *C. australis* adults was released at a field site near Merlin, Oregon (Balciunas and Villegas, 1999). Both flies apparently established. Examination of the voucher specimens retained from the overseas shipments received in quarantine suggested that the accidental release of *C. succinea* occurred just once (Balciunas and Villegas, 1999). Thus, the establishment rate of *C. succinea* in stage III is 100% (one of one).

## Stage IV – releases of domestic material

Three insects, *U. sirunaseva*, *B. orientalis* and *E. villosus*, built up high populations at field nursery sites and were distributed statewide through a distribution program. The program operated primarily through a series of workshops designed to train county biologists in the identification, collection and release of the biological control organisms. Based on the training received at the workshops, county biologists collected available biological control organisms from their release sites in stage III then returned to their county and released the insects at their own nursery sites. The new county sites would serve as sources for further distribution of insects within their counties. Training workshop sites began 2 to 3 years following initial release of foreign material in stage III. A total of 41,380 domestically produced *U. sirunaseva* was released at 163 locations, 80,290

**Table 1.** Summary for the implementation of seven seed head insects on yellow starthistle (*Centaurea solstitialis* L.) in California (USA). The values for stages III and IV represent the proportion of releases that successfully established.

| Species | Stage III (%) | Stage IV (%) | Stage V |
|---|---|---|---|
| *Urophora jaculata* (Rondani) | 0[a] | – | – |
| *Urophora sirunaseva* (Hering) | 100 | 87 | No |
| *Bangasternus orientalis* (Capiomont) | 100 | 92 | No |
| *Chaetorellia australis* (Hering) | 27[b] | 19 | No |
| *Eustenopus villosus* (Boheman) | 100 | 92 | Yes |
| *Larinus curtus* Hochhut | 100 | 25 | No |
| *Chaetorellia succinea* (Costa) | 100[c] | 100 | Yes |

[a] Turner *et al.* (1994).
[b] Turner *et al.* (1996).
[c] Balciunas and Villegas (1999).

*B. orientalis* at 371 locations, and 316,000 *E. villosus* at 1024 locations. Post-release monitoring at a subset of these locations estimated establishment at 87% (45 of 52, *U. sirunaseva*), 92% (118 of 128, *B. orientalis*) and 92% (119 of 129, *E. villosus*; Table 1).

Stage IV distribution of *C. australis* and *C. succinea* in California resulted from a release effort during 1994 through 1996 (Balciunas and Villegas, 1999). Encouraged by high populations of what was thought to be *C. australis* in southern Oregon, 9463 *Chaetorellia* adults were collected and released at 21 locations in California. It was during this release effort that the presence of *C. succinea* was discovered, and releases were discontinued. Post-release surveys of the 21 release sites recovered *C. australis* at four locations (establishment rate = 19%) and *C. succinea* at 21 locations (establishment rate = 100%).

The performance of the weevil, *L. curtus*, following establishment in stage III was disappointing as populations failed to increase and were recovered in low numbers several years afterward. A small number of field-collected adults from several release sites were found to be infested with a species of *Nosema*, a microsporidian parasite of the alimentary canal. A population of *L. curtus* in northern Oregon had readily established and built up high population numbers. Field samples later revealed no infection by *Nosema* at this location. It was thought that the microsporidian hindered population growth, so over 5600 adult *L. curtus* were collected from northern Oregon and released at 23 locations in California from 1997 to 1999. Post-release monitoring showed that the weevil established at five of 20 locations (establishment rate = 25%), but densities of the established populations remained extremely low.

## Stage V – local spread and population increase

Four species of seed head insects were recovered during an independent survey of yellow starthistle plants away from release sites in 2001 and 2002: *B. orientalis*, *U. sirunaseva*, *E. villosus* and *C. succinea*. All were found widespread throughout the state but in varying levels of abundance. Both *B. orientalis* and *U. sirunaseva* were recovered from 62% of the locations but occurred mostly in low numbers (usually <15% of seed heads attacked). In contrast, *E. villosus* was recovered at 80% of the locations, and its abundance ranged from 0% to 93% of the heads attacked. The fly, *C. succinea*, was recovered at 99% of the locations, and its abundance ranged from 0% to 96% of the heads attacked. The weevil, *L. curtus*, was not recovered. The fly, *C. australis*, was recovered in a follow-up survey where adult flies were reared from sampled seed heads; however, adult emergence rarely exceeded five flies per 100 heads.

Insect abundance, estimated as the number of heads attacked per sample, was interpolated between sample locations using a GIS system, and the resulting maps showed regional differences in abundance. For each insect species, the frequency distribution of abundance values was sorted by magnitude and divided into ten quantiles. The top quantile consisted of values in the top 10% of those recorded for the species. Areas of highest abundance were identified as those areas with interpolated values within the top quantile (Fig. 1). For *B. orientalis*, locations with abundance values above 18% heads attacked represented the top quantile, and these occurred in the mountain ranges of the most northern areas of California. For *U. sirunaseva*, locations with values above 9% attack rate represented the top quantile, and these areas were scattered within the northern and southern regions of the Coastal Mountain Range. For *E. villosus*, its top quantile consisted of locations with values above 41% attack rate, and these were observed throughout the mountains of northern California and along the foothills of the Sierra Nevada Mountains. Areas with the lowest abundance of *E. villosus* were observed in the San Joaquin and Sacramento Valleys. The fly, *C. succinea*, was the most common seed head insect recovered in the survey. Its top quantile consisted of abundance values above 70%. However, in comparison with the other seed head insects,

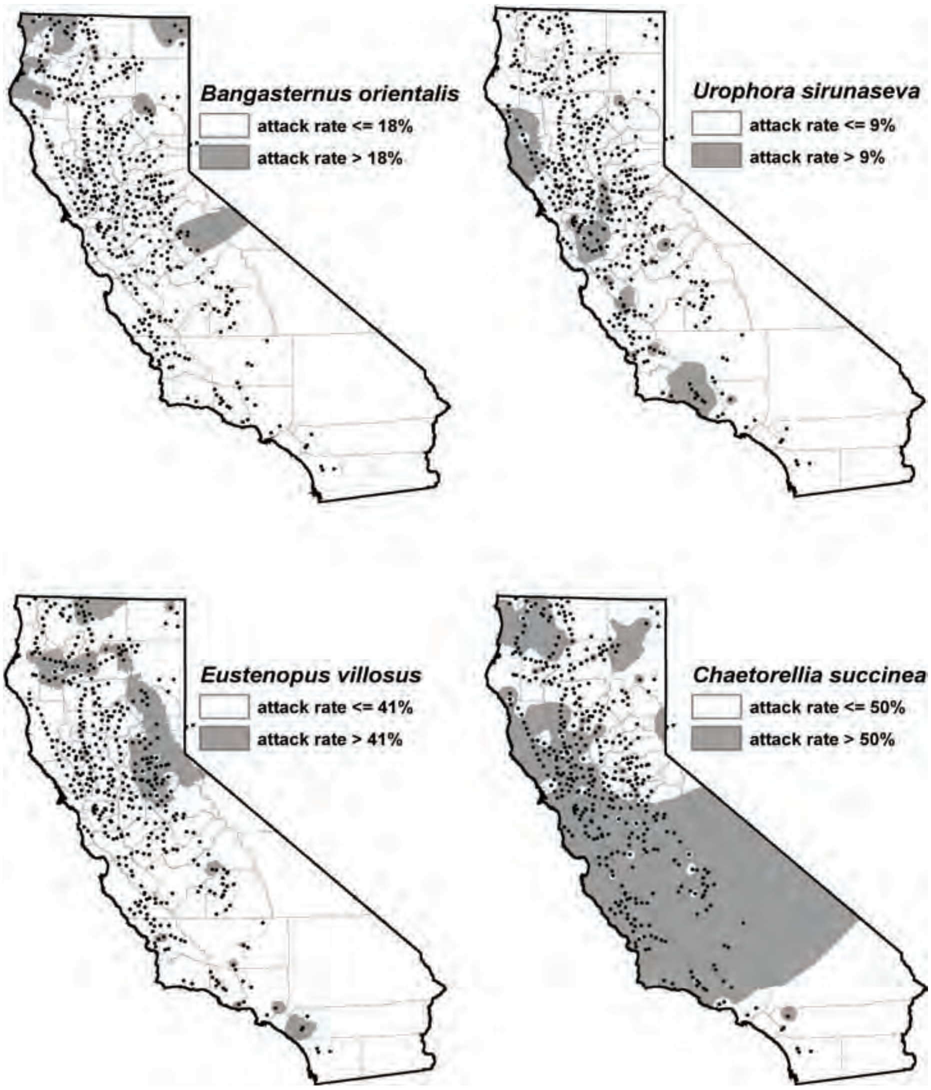


**Figure 1.** Plot of interpolation values for the four seed head insects recovered from *Centaurea solstitialis* L. in California during 2001–2002. All plots show areas of highest abundance as indicated by seed head attack rates in the top 10% of observed values (top quantile). The weevil *Bangasternus orientalis* and the fly *Urophora sirunaseva* rarely exceeded attack rates of 18% and 9%, respectively. The weevil *Eustenopus villosus* and the fly *Chaetorellia succinea* occurred in higher numbers (> 41% and 50%, respectively). Sample locations are indicated by the filled dots.

abundance values above 50% are considered high. For *C. succinea*, areas with interpolated abundance values above 50% occurred throughout central and southern California (Fig. 1).

## Discussion

The stage-based implementation model identified three main stages in the release and establishment of a biological control organism: establishment of foreign material from quarantine (stage III), establishment of domestic material (stage IV) and the increase and spread of the control organism throughout the region (stage V). Examination of establishment rates among stages shows that implementation of the six seed head insects intentionally introduced as biological control organisms was very successful. Five of six species established (83%) and establishment rates of the foreign material were 100% for four of five species. Collection and distribution of material produced domestically (stage IV) was also fairly successful with three of five species showing establishment rates above 87% throughout California. Interestingly, the accidentally introduced fly, *C. succinea*, also showed a high rate of establishment (100% in stages III and IV). However, the regional spread of the seed head insects at stage V was not as successful as only two species, *E. villosus* and *C. succinea*, appear to have built up populations approaching high densities

over large regional areas. The fly, *C. succinea*, appears to be the most successful organism with estimated attack rates greater than 50% over half of the area infested by yellow starthistle (Fig. 1). The weevil, *E. villosus*, was second in abundance with approximately 35% of the yellow starthistle infestation experiencing attack rates greater than 41%. None of the other insects appear to contribute much to the overall natural enemy pressure on this weed.

Several studies have identified propagule pressure as an important factor in the successful establishment of invading organisms. For example, Kolar and Lodge (2001) reviewed published studies on invasive species in an effort to identify attributes that might predict probable invaders. While the information they presented was limited to only a few taxa, one strong pattern was that successful establishment was positively related to propagule pressure. Propagule pressure, either number of individuals released or number of releases, is one factor that biological control workers have increasing control over due to improvements in rearing technology and faster, more efficient shipping abilities. For implementation efforts, establishment rates in stages III and IV would benefit from increases in the number of insects used. The only stage over which we appear to have little control is stage V. Reviews of past biological control projects (e.g. Hall and Ehler, 1979; Crawley, 1989; Simberloff, 1989; Coombs, 2004) have concentrated on establishment success in stages III and IV but provided little examination of projects in stage V. While establishment is an important component in the implementation of a biological control organism, ultimate success in controlling the target weed may reside in the attributes of control organisms that result in their ability to obtain high densities and regional spread. More research on the transition of biological control organisms to stage V may greatly improve our understanding of the attributes of an effective biological control agent and may lead to releases of more effective agents in the future.

## Acknowledgements

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# The ragweed leaf beetle *Zygogramma suturalis* F. (Coleoptera: Chrysomelidae) in Russia: current distribution, abundance and implication for biological control of common ragweed, *Ambrosia artemisiifolia* L.

**S.Ya. Reznik, I.A. Spasskaya, M.Yu. Dolgovskaya, M.G. Volkovitsh and V.F. Zaitzev**

*Summary*

The ragweed leaf beetle, *Zygogramma suturalis* F. (Coleoptera: Chrysomelidae), was introduced to Russia in 1978 against the common ragweed, *Ambrosia artemisiifolia* L. By 1985, it successfully acclimated and suppressed ragweed in the original release site and several neighbouring fields. However, because of crop rotation, its population density drastically decreased. In 2005 and 2006, we conducted selective quantitative sampling in Southern Russia. The results showed that the ragweed leaf beetle was distributed over an area of about 50,000 km$^2$ in Krasnodar territory, west of Stavropol' territory and south of Rostov province, i.e. most of the area heavily infested by ragweed in Russia. However, the average *Z. suturalis* population density was very low: approximately 0.001 adults per square metre (m$^2$) in crop rotations and approximately 0.1 adults/m$^2$ in more stable habitats, although in a few of the studied plots, up to 2–3 adults/m$^2$ were recorded. As for common ragweed, average percent cover in crop rotations and in stable habitats was approximately 1% and 40%, correspondingly. A detectable level of host plant damage (≥5%) was recorded only in a few plots. The observed spatial variation of the *Z. suturalis* population density was mostly determined by the host plant abundance. The last was strongly dependent on the stability of the habitat, being much higher in stable habitats. Thus, it is still possible that stable protected field nurseries could be a promising method of *Z. suturalis* propagation for biological control of ragweed in surrounding locations.

***Keywords***: weeds, biological control, post-release evaluation.

## Introduction

Common ragweed, *Ambrosia artemisiifolia* L., is one of the most noxious invasive weeds in Russia infesting agricultural fields, ruderal habitats etc. over more than 60,000 km$^2$ (Ul'yanova, 2003). In an attempt to control this weed, the ragweed leaf beetle, *Zygogramma suturalis* F., was introduced to Russia from the United States and Canada (Kovalev *et al.*, 1983). In 1978, about 1500 specimens were released in the vicinity of Stavropol'. By 1981, a significant increase in the *Z. suturalis* population density was recorded. In 1983, ragweed at the experimental release site was eliminated, and the ragweed leaf beetle began to spread over surrounding fields (Kovalev *et al.*, 1983). Thus, the initial phase of this introduction was a population explosion with more than a 30-fold yearly increase in number, up to 100,000,000 adults/km$^2$ in fields and up to 5000 adults/m$^2$ in aggregations (Kovalev, 1988). In some cases, a 'solitary population wave', i.e. a moving zone of ultra-high population density, was observed (Kovalev, 1988). Following these waves, common ragweed was exterminated locally very quickly, which allows one to expect highly efficient biological control of

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the weed (Kovalev, 1988; Goeden and Andres, 1999). From 1984 to 1986, a number of further releases were made in the south of European Russia and Ukraine, but the population wave was not subsequently observed, possibly because the insects were released in regularly exploited agricultural landscapes but not in special protected sites (Reznik *et al.*, 1994).

Later studies conducted in the area of the first release site have demonstrated that, in crop rotations, *Z. suturalis* population density drastically decreased despite a few patches with insect overcrowding and local weed control (Reznik *et al.*, 1994; Reznik, 1996). In these studies, regular estimations of the ragweed leaf beetle population density were restricted to a limited area in the environs of the initial release site. Now, almost 30 years after the initial release, data on *Z. suturalis* distribution and abundance are still fragmental (Ugryumov *et al.*, 1994; Polovinkina and Yaroshenko, 1999; Os'kin, 2002; Esipenko and Belikova, 2004).

At present, common ragweed invading Central and Western Europe poses a threat to the economy and human health by producing highly allergenic pollen in Austria, France, Italy, Hungary and Switzerland (Wittenberg, 2005). Analysis of the results of unsuccessful projects as well as of successful ones is important in improving the theory of biological control of weeds (Harris, 1993; Stiling, 1993; McFadyen, 1998; Müller-Schärer *et al.*, 2000; Raghu *et al.*, 2006). In addition, the 'planned invasion' of the ragweed leaf beetle can be considered as a model for predicting consequences of 'real' invasions (Ehler, 1998; Fagan *et al.*, 2002).

In 2005–2006, we conducted selective quantitative sampling over the whole area infested by *A. artemisiifolia* in Southern Russia. The aims of this study were to estimate *Z. suturalis* population densities in relation to environmental factors and to evaluate the impact of the phytophage on the targeted weed.

# Methods and material

## Methods

All studies were conducted between 15 July and 15 August, when *Z. suturalis* adults usually reach peak density (Kovalev *et al.*, 1983). In our earlier investigations (Reznik, 1993), 0.1 $m^2$ plots were used for exact determination of population densities by counting the beetles and measuring the plants. However, for the broad scale investigations, this method was found to be too time consuming. Later (Reznik *et al.*, 1994), the plant and the insect population densities were visually estimated with a scale of 0 to 5. But for the current study, fast visual estimation was not suitable, considering the low population density of the beetle. Thus, a new method was developed.

Plots with more or less uniform vegetation (particularly, ragweed abundance) separated from other plots by some natural borders (field boundary, road, forest belt etc.) were considered as sampling units. The size of the plot varied from 10 to 15 $m^2$ (isolated ragweed patches) to many hectares (agricultural fields). Usually, all plots along a randomly selected route were inspected.

For each plot, its square ($m^2$) and habitat type: agricultural field, field margin or ruderal (mostly roadsides) were recorded. The ragweed characteristics (percent cover and average height) were measured. *Z. suturalis* population density was measured by two methods. First, adults over a given square (one or several randomly selected transects of 1 m width and of total length depended on the size of the plot) were counted, and the mean number of adults per square metre was calculated. Second, sweeping with a standard net (along other randomly selected transects) was made, and the mean number of adults per ten sweeps was recorded. Impact on the targeted weed was evaluated visually by considering the relation (percentage) of the area eaten to primary leaf area. In addition, geographical coordinates of each plot, height above sea level, date of sampling and certain additional data were recorded.

## Material

Sampling was conducted from 2005 to 2006 in three main areas of Southern Russia heavily infested with common ragweed: Krasnodar territory, Stavropol' territory and Rostov province (Fig. 1). At nine locations (191 plots, total approximately 6.3 $km^2$) where the ragweed leaf beetle was relatively abundant, 760 adults were visually counted within 22,434 $m^2$ of transects, and 885 adults were collected by 6480 sweeps. In addition, a number of occasional records of *Z. suturalis* adults were made and used to determine a distribution range (Fig. 1). At six locations, the ragweed leaf beetle was not found, although 10–20 ragweed plots (total, 1000–3000 $m^2$) per location were carefully inspected.

## Statistical treatments

As could be expected, estimations of *Z. suturalis* population density made by counting and by sweeping closely correlated ($r = 0.78$, $n = 183$, Spearman rank correlation). Moreover, interrelations between data obtained by counting and by sweeping fit well ($r = 0.49$, Pearson correlation) with the linear regression $D_c = 0.14D_s$, where $D_c$ is the population density estimated by counting, and $D_s$ is that estimated by sweeping. Thus, the data obtained by two methods were combined with the formula $D_a = 1/2(D_c + 0.14D_s)$, where $D_a$ is the averaged *Z. suturalis* population density (adults per square metre). This integral parameter correlated well both with the results of counting and those of sweeping ($r = 0.95$ and $r = 0.91$, correspondingly, Spearman rank correlation). In eight cases, when sweeping was not made, $D_c$ was taken as $D_a$.

The ragweed population density was described not only by the height and percent cover but also by an integral characteristic, the ragweed abundance (the product of multiplication of the cover by the height). As the

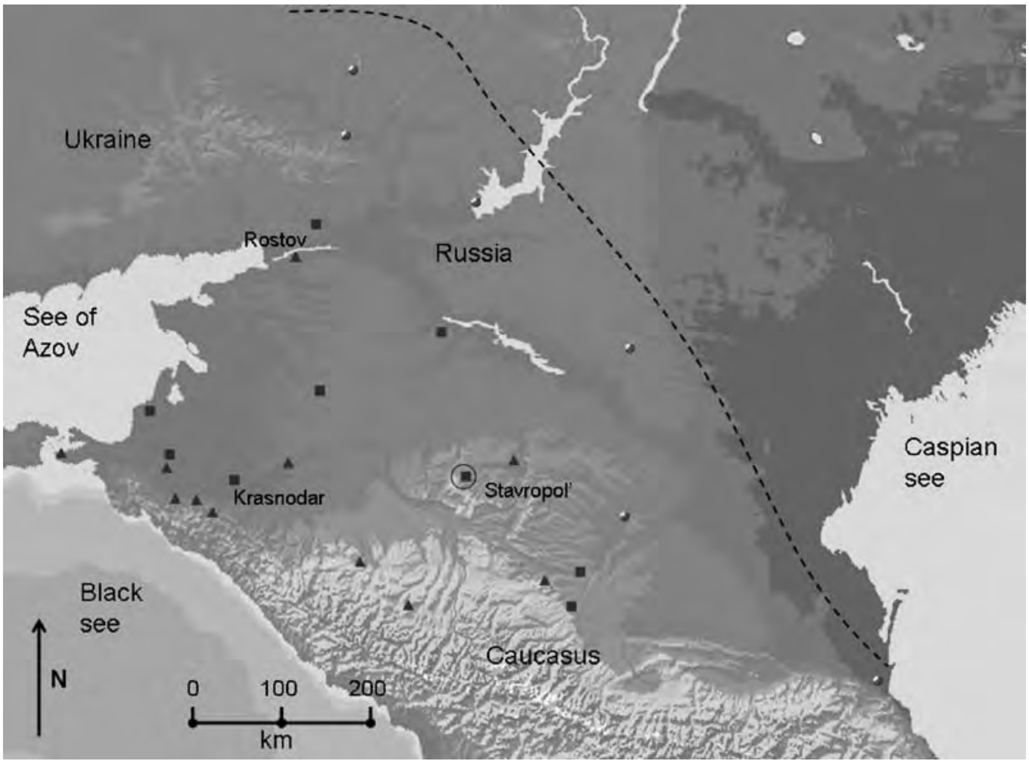


**Figure 1.** The geographical range of *Ambrosia artemisiifolia* and *Zygogramma suturalis* in Russia. Squares: Quantitatively studied locations; triangles: occasional records of *Z. suturalis* adults; circles: locations where *Z. suturalis* was not found; ring: the environs of the first release site, where the earlier studies (Reznik, 1993; Reznik *et al.*, 1994) were conducted; dash line: the approximate north-eastern boundary of the ragweed invasion.

distributions of *A. artemisiifolia* and *Z. suturalis* population densities were far from normal (Fig. 2), these data were transformed to decimal logarithms and then treated by analysis of variance (ANOVA), the Tukey test and linear regression analysis. Medians and quartiles of untransformed data were used as descriptive statistics. All calculations were made with SYSTAT 10.2.

## Results

Wide-scale sampling shows that at present, the ragweed leaf beetle is widely distributed in Krasnodar territory, west of Stavropol' territory and south of Rostov province (Fig. 1). *Z. suturalis* population density was significantly ($F = 3.4$, $n = 191$, $p = 0.001$) different among nine quantitatively studied locations and positively correlated with the average ragweed abundance ($r = 0.91$, $n = 9$, $p < 0.001$). Two-way ANOVA of the transformed data on six locations where all main types of habitats (fields, field margins and ruderal sites) were inspected ($n = 164$) showed that the ragweed leaf beetle population density was more dependent on type of habitat ($F = 8.6$, $p < 0.001$) than on location ($F = 3.1$, $p = 0.012$). As for the ragweed, its height was slightly dependent on location ($F = 2.5$, $p = 0.03$) and strongly dependent on type of habitat ($F = 11.7$, $p < 0.001$), while percent cover was dependent only on type of habitat ($F = 57.3$, $p < 0.001$) but not on location ($F = 1.4$, $p = 0.23$). The Tukey test showed that ragweed height, percent cover and *Z. suturalis* population density in agricultural fields were significantly ($p < 0.001$) lower that that in field margins and in ruderal sites, while the difference between field margins and ruderal sites was not significant ($p > 0.3$). Thus, for further data analysis, field margins and ruderal sites were pooled and considered as 'stable' habitats, in contrast to crop rotation.

The average (hereafter, medians, quartiles and sample size are given) population density of the ragweed leaf beetle was very low, 0.001 (0–0.03) adults per square metre in agricultural fields subjected to crop rotation ($n = 40$) and 0.1 (0.04–0.27) adults per square metre in stable habitats ($n = 151$). However, in few of the studied plots, *Z. suturalis* population density ranges up to two to three adults per square metre (Fig. 2). Considering rather high level of ragweed infestation: percent cover 1% (0–2) and 40% (20–60%), height 10 (0–30) and 50 (30–80) cm in crop rotation and in stable habitats, correspondingly, it is not surprising that a detectable level of host plant damage (≥5%) was recorded only in one agricultural field (sunflower) and in few stable plots with relatively high density of the phytophage (Fig. 3).

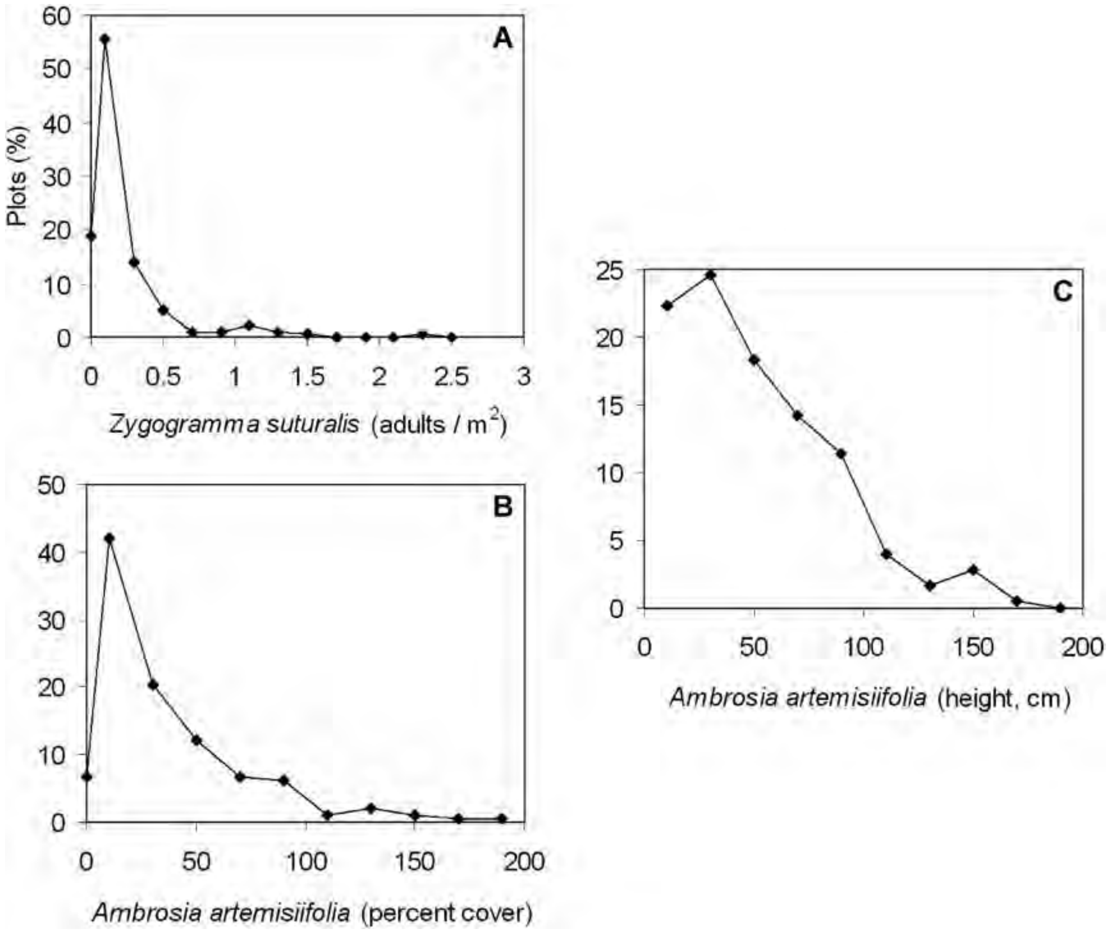


**Figure 2.** Distributions of the percent of studied plots according to the *Ambrosia artemisiifolia* and *Zygogramma suturalis* population densities which correspond to mean numbers of *Z. suturalis* adults per square metre ($n$ = 191), ragweed percent cover ($n$ = 191) and mean ragweed heights ($n$ = 175).

It is noteworthy that, in spite of the drastic difference between medians, general linear model analysis showed that *Z. suturalis* population density significantly ($p$ < 0.001) depended on the host plant abundance (cover multiplied by height) but not on the type of habitat ($p$ = 0.95). Parameters of linear regression of log-transformed *Z. suturalis* population density on log-transformed ragweed abundance almost coincided: $Y = 0.62X - 3.2$ ($r = 0.60$, $n = 40$, $p < 0.001$) for crop rotations and $Y = 0.59X - 3.1$ ($r = 0.40$, $n = 151$, $p < 0.001$) for stable habitats (see Fig. 3 for untransformed data).

## Discussion

First, our data suggest that *Z. suturalis* spreads practically over the whole area heavily infested by *A. artemisiifolia* in Russia (Ul'yanova, 2003), although it was not found in the less infested 'boundary zone'. Our observations suggest that in this zone, ragweed grows mostly in settlements and city environs, while in agricultural landscapes (including field margins), it is practically absent (unpublished data). It is well known (Maryushkina, 1986; Ul'yanova, 2003) that the northern limit of *A. artemisiifolia* geographical distribution in Russia is determined by the day length and temperature (ragweed is a typical 'short-day' plant) and the eastern limit by precipitation. Urban environments were shown to favor the ragweed establishment in the United States (Ziska *et al.*, 2006). Thus, it is possible that Russian settlements could also provide better conditions for the ragweed reproduction, i.e. more warm habitats in the northern boundary and more humid soil in the eastern boundary. Possibly, such a dispersed synanthropic distribution of ragweed in the boundary zone prevents or delays its colonization by the ragweed leaf beetle.

In the heavily infested zone, significant difference among studied plots and locations is most probably connected with uneven ragweed abundance but not with delayed colonization by the ragweed leaf beetle. An increase of the *Z. suturalis* population density with the *A. artemisiifolia* abundance was earlier recorded at smaller spatial scales (Reznik, 1993; Reznik *et al.*, 1994). As for the two components of the ragweed abundance, i.e.

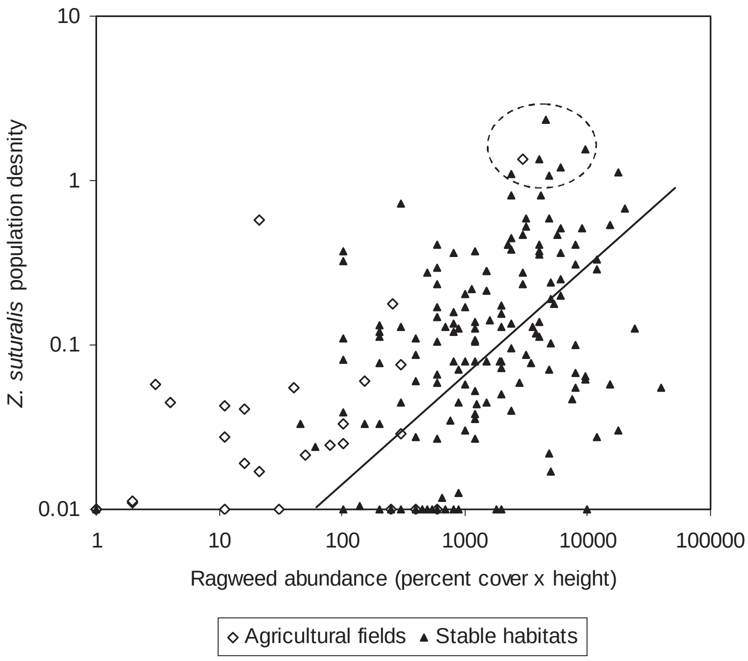


**Figure 3.** Relationship between *Ambrosia artemisiifolia* abundance (cover × height) and *Zygogramma suturalis* population density. *Each symbol* indicates one plot, an agricultural field subjected to crop rotation ($n$ = 40) or more stable habitat: field margin, roadside, ruderal site, etc. ($n$ = 151). *Regression line* ($Y = 0.61X - 3.1$, $r = 0.62$, $n = 191$, $p < 0.001$) is based on the log-transformed pooled data. *Dashed ellipse* indicates plots with a detectable rate of the ragweed damage (≥5% of the total leaf surface).

percent cover and average height, it is interesting that relatively stable habitats mostly differed from crop rotations not in ragweed percent cover but in its height. This could be explained by the fact that mowed ragweed can regrow quickly, producing many leaves on short stems. Hence, at the same percent cover in stable habitats, ragweed is much higher.

Note that *Z. suturalis* population densities recorded in 2005 to 2006 were lower than those observed during our previous investigations (Reznik, 1993; Reznik *et al.*, 1994; Reznik, 1996). As for the data obtained by other authors, the comparison is hampered by the fact that, very often, not average values but upper limits were published. Maximal *Z. suturalis* population density recorded in Stavropol' and Krasnodar territories at the end of the 1990s was six and 50–70 adults per square metre, respectively (Polovinkina and Yaroshenko, 1999; Os'kin, 2002), which is similar to our data obtained in 1988–1989 (Reznik *et al.*, 1994) but much higher than the results of the present. One of the recent papers (Esipenko and Belikova, 2004) also pointed out a significant decrease in the *Z. suturalis* population density. This assertion is indirectly supported by the fact that in several regions of Krasnodar territory, where in 1993 the ragweed leaf beetle population density ranged up to 400 adults/$m^2$ (Ugryumov *et al.*, 1994), in 2003, only 166 adults were collected for phenetic analysis (Esipenko and Savva, 2004). It is not clear if this decline in population density is connected with recent climatic changes, with increasing impact of natural enemies, or with some other factors (Esipenko and Belikova, 2004).

Thus, our results suggest that, by now, the ragweed leaf beetle had spread throughout most of its potential area, and further expansion is unlikely. The observed spatial variation of *Z. suturalis* population density seems to be mostly determined by the host plant abundance. The last is strongly dependent on the stability of the habitat, being much lower in crop rotation fields. In overwhelming majority of inspected populations, the impact on the targeted weed is negligible. However, having regard to the spectacular success achieved in the permanent experimental plot from 1983 to 1985 (Kovalev, 1988), it is still possible that stable protected field nurseries could be a promising method of *Z. suturalis* propagation for biological control of ragweed in surrounding areas.

As already highlighted by the biological invasion theory, we conclude that, in spite of certain prerequisites for invasion (abundant food resources, absence of competitors, specific parasitoids and predators), very strong population explosion recorded during first years after introduction finally resulted in wide geographic distribution but low mean population density and negligible ecological role of an alien insect species.

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# Long-term field evaluation of *Mecinus janthinus* releases against Dalmatian toadflax in Montana (USA)

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*Summary*

The toadflax stem mining weevil, *Mecinus janthinus* Germar, was first released in the United States in Montana, in 1996. This agent has now become established to varying degrees after subsequent releases made at sites throughout the state. Multiple releases of *M. janthinus* have presented researchers with a unique opportunity to evaluate the efficacy of this agent in diverse habitats and under a variety of environmental conditions. The results presented in this paper summarize findings from long-term field data, illustrating not only the impact of *M. janthinus* on the target weed, Dalmatian toadflax, *Linaria dalmatica* (L.) P. Mill., but also on correlated plant community dynamics. These results additionally provide a valuable means to compare and contrast the biotic response and control efficacy of this agent at both a regional and sub-continental scale.

***Keywords***: *Linaria*, efficacy, plant community response.

## Introduction

Dalmatian toadflax, *Linaria dalmatica* (L.) P. Mill. (Scrophulariaceae) (USDA, NRCS 2007), is an invasive short-lived perennial forb of Mediterranean origin (Alex, 1962). Intentionally introduced to North America as an ornamental plant, *L. dalmatica* is now widespread and has effectively become naturalized through multiple introductions over time (Lajeunesse, 1999). Aspects of the species' life history and morphology, including a root system characterized by a long, well-developed taproot and extensive lateral roots, dual modes of reproduction through seed and vegetative root buds, coupled with a high rate of seed production and long term seed viability undoubtedly contribute to its dominance in disturbed range and forested lands (Robocker, 1974; Vujnovic and Wein, 1997).

Herbicide treatment of Dalmatian toadflax is hampered by two factors: (1) the species' deep root system necessitates precise timing of herbicide application when root carbohydrate reserves are low and the plant is therefore more susceptible to chemical translocation and impact (Robocker *et al.*, 1972) and (2) the protective waxy leaf coating resists herbicide penetration (De Clerck-Floate and Miller, 2001). Chemical control of Dalmatian toadflax is expensive due to the typically large acreages affected. Additionally, repeated herbicide applications are frequently necessary in western US water-limited habitats because each precipitation event has the potential to stimulate Dalmatian toadflax regeneration from fire-resistant rootstocks and characteristically large seedbanks (Zouhar, 2003).

Classical biological control of Dalmatian toadflax in North America was initiated in the late 1960s. To date, eight exotic agent species targeting the flowers, stems, foliage or roots of Dalmatian toadflax have been released or, in the case of adventitiously introduced agents, redistributed, and have established to varying degrees in North America (Smith, 1956; Harris and Carder, 1971; Harris, 1984; De Clerck-Floate and Harris, 2002; McClay and De Clerck-Floate, 2002).

Ten years of research results indicate that biological control of Dalmatian toadflax is feasible, particularly with the stem-boring weevil, *Mecinus janthinus* Germar, the most recently approved agent for control of Dalmatian toadflax. *M. janthinus* has established and

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proliferated on many sites throughout western Canada and the United States and is probably the best agent currently available for managing Dalmatian toadflax (Harris *et al.*, 2000; Nowierski, 2004), although population growth is impeded by high levels of overwintering mortality (De Clerck-Floate and Miller, 2002) and site-specific climatic factors (McClay and Hughes, 2007).

Advocates of weed biological control tout this approach for its specificity. Biological control is, in general, a significantly more gradual process than alternative control approaches such as herbicide application. Both of these characteristics facilitate control of the target weed without selectively or intensively influencing immediate changes in the wider vegetation community. Reductions in invasive species such as Dalmatian toadflax should result in the restoration of desirable species, especially those within the target weed's functional group, in the vegetation community. Unfortunately, herbicide treatments frequently result in the desired decrease in Dalmatian toadflax, followed by either an increase in bare ground or replacement with an undesirable species that poses an even greater environmental risk. For instance, cheatgrass (*Bromus tectorum* L.) and exotic, invasive knapweeds commonly invade areas where Dalmatian toadflax has been treated with herbicide; cheatgrass is known to significantly alter vegetation community dynamics and fire cycles (Whisenant, 1990; Billings, 1994), while spotted knapweed (*Centaurea maculosa* Lam.) is regarded as an allelopathic species (Bais *et al.*, 2003). The purpose of this long-term evaluation was to determine if indicators of improved vegetation community dynamics, specifically increased cover of desirable vegetation, can be correlated with the release of the biocontrol agent *M. janthinus*.

## Methods and materials

Vegetation data were recorded as early as 4 years before the release of *M. janthinus* (1992) and continued through 2007 at seven release sites located throughout Montana (see Table 1 for site-specific details). The 'Bison Range' site (US Fish and Wildlife Service and Bureau of Indian Affairs) is located in the northwest near Pablo on Flathead Indian Reservation tribal lands adjoining the National Bison Range; three sites, 'Canyon Ferry' (Bureau of Reclamation), 'Elkhorns' (Bureau of Land Management) and 'Mount Helena' (City of Helena Parks and Recreation) are located in the southwest, near the city of Helena, MT; the 'Crow' site (Bureau of Indian Affairs) is located south-centrally on the Crow Reservation, near Lodgegrass, MT; 'Hardy Bridge' (Bureau of Land Management) is located south of Great Falls, MT; and the eastern-most 'Melstone' site (Bureau of Land Management) is located on private ranch land bordering public lands, near the town of the same name.

The initial releases of *M. janthinus* in Montana were made on the Crow and Elkhorns sites in 1996 with limited numbers of adult weevils. *M. janthinus* was first released on the Canyon Ferry and Bison Range sites in 1997, on the Mount Helena site in 1999, and at the Melstone and Hardy Bridge sites in 2000. Permanent, paired 20-m vegetation monitoring transects were initially established to run through the densest local infestations of Dalmatian toadflax at each site. Vegetation data were recorded annually from fixed points at 1-m intervals along each transect. Vegetation attributes were reported from 0.10 $m^2$ Daubenmire frames placed on fixed sample points, with a total of 40 samples taken annually at each site.

Data collected from each sample frame included counts of Dalmatian toadflax plants and individual stems; the stems were categorized and counted as mature or immature stems. The criterion used to identify mature stems was obvious evidence of flower buds or actual flowering. Stem counts were taken in addition to plant densities because Dalmatian toadflax is not easily or accurately censused on a per-plant basis without destructive excavation. In addition, percent cover of Dalmatian toadflax only and of all forbs other than Dalmatian toadflax was recorded. The remaining area within the sample quadrat not accounted for by one of the vegetation life forms was categorized as non-vegetation cover and included the total area covered by litter, rock and soil. For each plant parameter or vegetation category, mean values for data collected for the year of permanent transect establishment were compared with those for the most recent year of sampling (2006). Mean comparisons were made for each site using a Student's *t* test (Dixon and Massey, 1969).

As part of a non-destructive indicator sampling strategy, 50 randomly selected dead stems from the previous year were collected at each site near, but external to, the monitoring transects. Collecting 'dead' Dalmatian toadflax stems provides a means for evaluating *M. janthinus* establishment and overwintering mortality without influencing weed or insect population trajectories within the monitoring transects. Stems were then split with a scalpel to estimate the number of live or emerged adult weevils. Empty *M. janthinus* chambers are a reliable 1:1 indicator of successful adult *M. janthinus* emergence (R. DeClerck-Floate, personal communication). Weevil mortality was also included as count data and encompassed non-emerged adults, larvae and pupae. Mean comparisons, with $\alpha = 0.05$, were made between emerged adults and dead individuals at each site using a Student's *t* test (Dixon and Massey, 1969).

## Results

Overwintering mortality continues to be a concern for Montana *M. janthinus* populations. Estimates of dead

**Table 1.** Comparison of mean pre- and post-release vegetation attributes (±SE) for transect samples taken at multiple *Mecinus janthinus* release sites.

| Site | Year | Transect | No. Dalmatian toadflax stems | Dalmatian toadflax % cover | Other forbs % cover | Bare substrate % cover |
|---|---|---|---|---|---|---|
| Bison Range | 1992 | 1 | 4.10 ± 0.41 | 8.80 ± 1.11 | 5.50 ± 1.05 | 74.30 ± 2.92 |
| Bison Range | 2006 | 1 | 0.10 ± 0.07 | 0.50 ± 0.34 | 14.00 ± 1.29 | 72.50 ± 1.83 |
| *P* values | | | 0.0027 | ≤0.0001 | ≤0.0001 | 0.5596 |
| Bison Range | 1992 | 2 | 5.00 ± 0.45 | 15.75 ± 1.71 | 0.25 ± 0.25 | 49.75 ± 3.95 |
| Bison Range | 2006 | 2 | 0.35 ± 0.17 | 1.00 ± 0.46 | 9.00 ± 1.39 | 81.25 ± 1.58 |
| *P* values | | | 0.0001 | ≤0.0001 | ≤0.0001 | ≤0.0001 |
| Canyon Ferry | 1996 | 1 | 2.90 ± 0.49 | 8.30 ± 2.08 | 6.25 ± 1.73 | 70.95 ± 3.26 |
| Canyon Ferry | 2006 | 1 | 6.15 ± 0.88 | 12.00 ± 1.79 | 4.25 ± 0.41 | 70.75 ± 2.21 |
| *P* values | | | 0.0100 | 0.2288 | 0.1654 | 0.9629 |
| Canyon Ferry | 1996 | 2 | 5.10 ± 0.69 | 16.50 ± 2.21 | 4.25 ± 1.27 | 61.25 ± 3.70 |
| Canyon Ferry | 2006 | 2 | 2.45 ± 0.41 | 4.25 ± 0.83 | 11.50 ± 2.15 | 72.75 ± 2.42 |
| *P* values | | | 0.0164 | ≤0.0001 | ≤0.0001 | 0.0073 |
| Crow | 1993 | 1 | 5.35 ± 1.09 | 9.80 ± 2.25 | 2.75±1.11 | 65.60 ± 2.83 |
| Crow | 2006 | 1 | 0.05 ± 0.05 | 0.25 ± 0.25 | 7.00 ± 0.76 | 82.25 ± 1.38 |
| *P* values | | | ≤0.0001 | ≤0.0001 | 0.1071 | ≤0.0001 |
| Crow | 1992 | 2 | 7.15 ± 1.25 | 18.45 ± 2.89 | 2.05 ± 0.74 | 61.50 ± 2.70 |
| Crow | 2006 | 2 | 0.00 ± 0.00 | 0.00 ± 0.00 | 9.75 ± 1.12 | 73.25 ± 1.82 |
| *P* values | | | ≤0.0001 | ≤0.0001 | 0.0113 | 0.0039 |
| Elkhorns | 1992 | 1 | 4.40 ± 0.60 | 12.15 ± 2.21 | 0.95 ± 0.54 | 53.55 ± 3.90 |
| Elkhorns | 2006 | 1 | 0.55 ± 0.20 | 2.00 ± 0.67 | 5.75 ± 0.66 | 72.50 ± 1.68 |
| *P* values | | | 0.0283 | ≤0.0001 | ≤0.0001 | ≤0.0001 |
| Elkhorns | 1992 | 2 | 2.80 ± 0.59 | 10.75 ± 2.30 | 2.15 ± 0.86 | 65.00 ± 2.55 |
| Elkhorns | 2006 | 2 | 1.35 ± 0.44 | 2.75 ± 0.92 | 4.25 ± 0.98 | 76.25 ± 2.20 |
| *P* values | | | 0.4108 | ≤0.0001 | 0.0435 | 0.0001 |
| Hardy Bridge | 2002 | 1 | 2.80 ± 0.73 | 9.50 ± 2.35 | 3.75 ± 1.02 | 60.75 ± 2.95 |
| Hardy Bridge | 2006 | 1 | 0.55 ± 0.28 | 1.00 ± 0.46 | 11.50 ± 2.57 | 64.50 ± 2.56 |
| *P* alues | | | 0.0123 | 0.0005 | 0.0014 | 0.3363 |
| Hardy Bridge | 2002 | 2 | 3.10 ± 0.50 | 12.25 ± 1.56 | 8.50 ± 1.31 | 58.50 ± 1.59 |
| Hardy Bridge | 2006 | 2 | 0.20 ± 0.16 | 0.50 ± 0.34 | 4.50 ± 0.88 | 72.00 ± 1.37 |
| *P* values | | | ≤0.0001 | ≤0.0001 | 0.0028 | ≤0.0001 |
| Melstone | 2000 | 1 | 2.30 ± 0.57 | 11.00 ± 2.39 | 12.25 ± 1.72 | 74.00 ± 2.66 |
| Melstone | 2006 | 1 | 2.00 ± 0.70 | 5.50 ± 1.95 | 7.00 ± 0.84 | 63.50 ± 3.29 |
| *P* values | | | 0.6923 | 0.0206 | 0.0013 | 0.0145 |
| Melstone | 2000 | 2 | 2.40 ± 0.39 | 12.25 ± 2.22 | 8.50 ± 1.50 | 73.25 ± 2.67 |
| Melstone | 2006 | 2 | 2.40 ± 0.54 | 5.50 ± 1.25 | 7.75 ± 1.12 | 74.50 ± 2.35 |
| *P* values | | | 1.0000 | 0.0013 | 0.6038 | 0.7054 |
| Mount Helena | 1992 | 1 | 7.60 ± 1.36 | 14.95 ± 2.09 | 6.35 ± 1.21 | 46.70 ± 5.32 |
| Mount Helena | 2006 | 1 | 2.60 ± 0.63 | 8.75 ± 1.58 | 8.75 ± 1.49 | 68.50 ± 3.12 |
| *P* values | | | 0.0013 | 0.0093 | 0.2483 | ≤0.0001 |
| Mount Helena | 1992 | 2 | 9.90 ± 1.23 | 16.35 ± 1.74 | 3.70 ± 1.61 | 43.10 ± 4.03 |
| Mount Helena | 2006 | 2 | 1.65 ± 0.52 | 5.00 ± 1.70 | 10.75 ± 1.42 | 76.75 ± 2.44 |
| *P* values | | | 0.0399 | ≤0.0001 | 0.0014 | ≤0.0001 |

individuals per stem generally outnumbered those of live adults that successfully emerged (Table 2). *M. janthinus* mortality was statistically greater than emergence for at least 1 year of the study at all sites except 'Bison Range' and Hardy Bridge', while mortality was significantly lower than emergence only at 'Bison Range' in 2003. Mortality was as much as fourfold higher than adult emergence at certain sites in some years.

At five of seven study sites, data from both transects showed reductions in toadflax density compared to pre-release levels (Table 1). No significant change was recorded at the Melstone site, while transect 1 at Canyon Ferry showed a significant increase in toadflax density. Percent cover of Dalmatian toadflax was significantly lower at all sites with the exception of transect 1 at Canyon Ferry. We found that percent cover of

**Table 2.** Estimates of mean (±SE) number of alive and 'dead' *Mecinus janthinus* per *Linaria dalmatica* stem, based on a random sample of 50 stems collected adjacent to release monitoring transects. Alive individuals refer to empty pupal cells, 'dead' individuals refer to the sum of non-emerged adults, pupae and larvae. Means marked with an *asterisk* are significantly different from the other in the same row at $P < 0.05$.

| Site | Year | Number of individuals | |
|---|---|---|---|
| | | Alive | 'Dead' |
| Bison Range | 2003 | 0.96 ± 0.18* | 0.50 ± 0.12 |
| | 2004 | 0.26 ± 0.10 | 0.28 ± 0.16 |
| | 2005 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | 2006 | 0.02 ± 0.02 | 0.08 ± 0.05 |
| Canyon Ferry | 2003 | 0.08 ± 0.08 | 0.06 ± 0.03 |
| | 2004 | 0.06 ± 0.04 | 0.16 ± 0.07 |
| | 2005 | 0.02 ± 0.02 | 0.12 ± 0.07 |
| | 2006 | 0.04 ± 0.03 | 0.36 ± 0.11* |
| Crow | 2003 | 0.66 ± 0.13 | 1.20 ± 0.18* |
| | 2004 | 1.68 ± 0.26 | 2.50 ± 0.44* |
| | 2005 | 2.08 ± 0.41 | 3.64 ± 0.52* |
| | 2006 | 1.80 ± 0.25 | 1.70 ± 0.26 |
| Elkhorns | 2003 | 0.02 ± 0.02 | 0.10 ± 0.04* |
| | 2004 | 0.00 ± 0.00 | 0.02 ± 0.02 |
| | 2005 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | 2006 | 0.20 ± 0.09 | 0.80 ± 0.25* |
| Hardy Bridge | 2003 | 0.08 ± 0.03 | 0.10 ± 0.05 |
| | 2004 | 0.00 ± 0.00 | 0.02 ± 0.02 |
| | 2005 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | 2006 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Melstone | 2004 | 1.18 ± 0.25 | 3.18 ± 0.44* |
| | 2005 | 0.78 ± 0.14 | 1.06 ± 0.18 |
| | 2006 | 0.32 ± 0.08 | 1.44 ± 0.23* |
| Mount Helena | 2003 | 0.48 ± 0.10 | 0.78 ± 0.15 |
| | 2004 | 0.88 ± 0.19 | 2.14 ± 0.47* |
| | 2005 | 0.94 ± 0.17 | 1.50 ± 0.25* |
| | 2006 | 0.70 ± 0.16 | 2.94 ± 0.40* |

forbs other than Dalmatian toadflax increased on 10 of the 14 total transects, and the increase was significant in eight cases. Non-vegetation cover increased significantly on nine transects, remained unchanged on four transects and decreased significantly only on transect 1 at Melstone.

## Discussion

The survival of *M. janthinus* was quite low at these sites over the years studied, and this may have reduced potential population growth. It has been reported that extreme temperatures (De Clerck-Floate and Miller, 2002; McClay and Hughes, 2007) and reduced snow cover (De Clerck-Floate and Miller, 2002) reduce survival and population growth of *M. janthinus*. Persistent drought conditions in Montana from 1997 through 2004 undoubtedly affected vegetation dynamics on all sites. The increase in unvegetated area would limit snow retention, which could increase overwintering mortality due to lower temperatures. Although exact environmental conditions have not been determined at these study sites, the locations do experience temperature and precipitation patterns similar to those described for Canada (De Clerck-Floate and Miller 2002), but percent cover of Dalmatian toadflax was lower in Montana, even before biological control was initiated.

However, the general trend towards increased cover of forbs at these sites indicates that climatic conditions alone were not driving the decrease in Dalmatian toadflax density and cover observed at many of our study sites. This may be attributable to biological control altering the overall dynamics of the entire forb community by reducing competition from Dalmatian toadflax. In addition, the increased proportion of forbs that was correlated with an increase in unvegetated area at most sites does suggest that, under comparative environmental extremes, biological control may offer an alternative to protracted non-target impacts from non-selective herbicide.

In addition, we also observed that increased grazing pressure on toadflax appeared to be correlated with drought conditions, especially in late summer when the weed was one of few remaining undesiccated species available. Cattle and wildlife grazing at all sites may have significantly impeded population buildup of *M. janthinus*. Grazing of succulent stems removes developing immature weevils from the population, resulting in a likely reduction in the following season's reproductive population. Weevil populations rebounded when drought conditions eased, and adult weevils became numerous enough to be collected for redistribution on three sites in 2006, suggesting that desiccation before and during winter has also probably been a major impediment to population increase of *M. janthinus* in Montana.

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# Post-release evaluation of invasive plant biological control agents in BC using IAPP, a novel database management platform

**S.C. Turner**

*Summary*

When faced with the invasion of alien plant species, the government of British Columbia (BC) uses the principals of Integrated Pest Management: prevention, early detection rapid response, inventory surveys and mechanical, chemical and biological control treatments. The BC government's Invasive Alien Plant Program (IAPP) application houses the data for management of invasive alien plants, including planning, inventory, mechanical, chemical and biological control, the monitoring of each of these activities and biological control agent dispersal. The IAPP Application is structured to track sites and their characteristics as geographic locations. Multiple invasive species with multiple surveys can be entered for a single site. This allows recording of the change in the invasive plant community over time and the level of success of treatment efforts. A compilation of this data allows assessment of the current set of biological control agents for target plant species. By comparing the spread of Dalmatian toadflax, *Linaria dalmatica* L. Miller, to the habitat requirements of its biological control agents, it is possible, using IAPP, to determine whether sufficient, suitable agents exist in the province or whether additional screening of agents must be pursued.

***Keywords***: Dalmatian toadflax, biological control agent habitat requirements, spatial mapping.

## Introduction

The Crown public lands of British Columbia (BC) consist of approximately 885,600 km$^2$ or 93% of the province (BC Ministry of Sustainable Resource Management, 1997). When faced with the invasion of alien plant species, BC employs the principals of Integrated Pest Management: prevention, early detection rapid response (EDRR), inventory surveys and a multitude of treatment tools including mechanical, chemical and biological control. For example, upon initial sightings of an invasive alien species, mechanical treatment may be used when the infestation is of a size to be managed by this method or when the plants reside in a herbicide-restricted area. Herbicide treatment is a necessary and effective tool that is used judiciously.

British Columbia's legislation for herbicide use, administered and enforced by the Ministry of Environment, is protective of the natural environment. For example, the pesticide-free zone required around or along bodies of water, dry streams and classified wetlands is 10 m, and BC has no herbicides registered for use against invasive plants in water. When an invasive alien species has spread to an area where repeated spraying is not economical, a containment strategy is employed. Under this strategy, a perimeter boundary around an infestation is established, and no mechanical or chemical treatment is carried out within the perimeter except in special circumstances. Any invasive plant found beyond the boundary is aggressively treated.

Once an invasive species has spread beyond the ability to manage it with mechanical or chemical treatment methods, the area is considered to require restoration. Restoration of infested areas is pursued through the use of good resource management practices and the encouragement of healthy biological control agent populations, where available.

BC Ministry of Forests and Range (MFR) staff interact with scientists in the Canadian and international community pursuing biological control agents

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for invasive alien plant species of concern. Consortia are formed, comprised of funding agencies, national researchers and influential stakeholders that plan and pursue biological control research. The invasive plant's country of origin is investigated for insects and pathogens that are found to attack the target plant species. Through cooperative efforts, BC funds research to ensure that biological control agents are specific to the target plant, that is, they will not inflict major damage to other Canadian plants of economic or environmental importance.

When new biological control agents have passed the screening process and approval for importation has been obtained, they are shipped into the country, passed through the Agriculture and Agri-Food Canada quarantine facility at Lethbridge, Alberta and received in British Columbia. Once in British Columbia, the agents are released at pre-determined sites for optimum establishment. These sites are determined from information on the agent's native habitat, investigation into the provincial government's Invasive Alien Plant Program (IAPP) application and field investigation. The release sites are in turn tracked in the IAPP Application.

The IAPP Application (http://www.for.gov.bc.ca/hfp/invasive/index.htm), launched in fall 2005, is a web-based Oracle application that houses data associated with the integrated weed management processes. Information is entered into the IAPP application for individual sites including their geographical characteristics and invasive plant species. Data on multiple invasions from surveys that assess the management strategies can be included for each site. This creates a record of the change in the invasive plant populations over time and allows evaluation of the level of success of management practices.

The IAPP application consists of two components: a password-protected Data Entry module and a Map Display module that is accessible to the public. Collaborating organizations may be given access to IAPP to record information. The information is visible to all participants but is protected from manipulation. Efforts to manage invasive alien plants can, therefore, be coordinated among all participants.

For applied biological control, sites are chosen where there is a minimal chance of conflicting treatments occurring and after considering habitat-requirement information. The latter information is obtained from screening and petition documents, field staff and from the Biogeoclimatic Ecosystem Classification (BEC) system, which is viewed as a layer in the IAPP application. The BEC (http://www.for.gov.bc.ca/hre/becweb/) system groups ecosystems into hierarchical classifications. A unit within the BEC system is defined as a particular plant community and its associated physiography, soil and climate (Meidinger and Pojar, 1991).

Biological control agent releases are recorded into the IAPP's Data Entry module. The sites are viewed spatially in IAPP's Map Display as polygons of various sizes to represent, from small to large: a Universal Transverse Mercator (UTM) co-ordinate; a 'protected' location for biological control agents that have yet to establish; and the size and shape of an invasive plant infestation. Thereafter, a site is monitored to determine if the agent has established and whether any change has occurred in the density, area or distribution of the target invasive plant.

Additionally, the IAPP application can house biological control agent dispersal information. Dispersal information is used to determine locations for the collection and redistribution of biological control agents.

Altogether, the spatial information displayed in the IAPP application allows for an increased understanding of the habitat requirements of particular biological control agents. Predictions can be made of the agent's ability to colonize different habitat types and locations. This can give invasive plant managers and scientists direction for pursuing new biological control agents and the habitat types they are required to fill. An example of how the IAPP application is being used can be seen in the case of the invasive Dalmatian toadflax, *Linaria dalmatica* L. Miller, and its complement of biological control agents, in particular the stem weevil, *Mecinus janthinus* Germar, and the seed weevil, *Rhinusa antirrhini* (Paykull).

Dalmatian toadflax is a short-lived perennial herb that was introduced as an ornamental in the United States in 1894. It originates from the Mediterranean region, from Yugoslavia to Iran (Robocker, 1974). Dalmatian toadflax spreads by creeping root stock and seeds, each plant potentially producing up to 500,000 seeds (Robocker, 1974) that are dispersed mainly by wind and browsing animals. Mature plants are 60 to 120 cm tall. The stems, several per plant, are smooth and light-green, and the flowers are 'snapdragon' shaped (Powell et al., 1994). The plant is toxic to livestock, and cattle tend to avoid grazing on infested pastures (Mason and Huber, 2002).

Dalmatian toadflax is a stress-tolerant plant able to grow in conditions of low temperature, coarse soils and summer drought. It exists in the BC BEC zones of Bunchgrass, Ponderosa pine, Interior Douglas-fir, Interior cedar-hemlock, Coastal Douglas-fir, Coastal Western hemlock, Montane spruce and the Sub-boreal spruce.

Efforts to acquire biological control agents for BC Dalmatian toadflax began in the 1960s (Mason and Huber, 2002). Since then, seven agents have been screened, and these have been released or have arrived adventively in the province. In particular, *M. janthinus* (stem-boring weevil) was introduced in 1991, and *R. antirrhini* (seed-feeding weevil) was introduced in 1993. These two species are actively being redistributed across the province.

In its native (European) distribution, *M. janthinus* was recorded residing in a wide range of habitat types. It has been recorded from just below the subalpine zone

in the Alps to the 'maritime lowlands in western and northern France and northern Germany' to the 'Mediterranean climate of the Rome area in Italy' and to the 'subcontinental, summer-dry regions of eastern and southern Yugoslavica and south-western Russia' (Jeanneret and Schroeder, 1992). It is also believed to exist in 'other parts of southern Germany, in Austria, Hungary and the Balkans' (Jeanneret and Schroeder, 1992).

Based on its native distribution, *M. janthinus* was expected to establish in all habitats where yellow toadflax, *Linaria vulgaris* L., and Dalmatian toadflax exist in North America between the latitudes of 40° and 52°. In Canada, this would include south-central BC, southern Alberta and Saskatchewan, as well as the maritime areas in eastern Canada. In the United States, this would include Washington, Oregon, Montana, northern California and the maritime areas of the eastern USA (Jeanneret and Schroeder, 1992). It has also been stated by Powell et al. (1994) that *M. janthinus* prefers hot, dry conditions usually found in grasslands or open forest with grasslands.

Typically, *M. janthinus* attacks Dalmatian toadflax by ovipositing single eggs into holes that the female chews in the stem. The holes are sealed and, in turn, covered by callouses that appear as tiny round blemishes on the stem (Jeanneret and Schroeder, 1992). Multiple eggs may be laid into a single stem, and the larvae feed in the stem, disrupting the flow of nutrients and causing the cessation of flowering. More than 100 weevils have been found in a single large stem (Mason and Huber, 2002). This activity takes place from spring until mid-summer. Infestations of Dalmatian toadflax are noticeably affected by large populations of *M. janthinus*, for example in the Lac du Bois grassland park near Kamloops, BC, where the weevil was released in 1997 (Figs. 1 and 2).

The native distribution of *R. antirrhini* is recorded as central and southern Europe, the Mediterranean region and the Caucasus (Groppe, 1992). Without in-depth knowledge of the insect's habitats in these regions, it is difficult to determine which habitats in BC might be conducive to either or both agents.

Females of *R. antirrhini* oviposit into the flower carpel in June (Groppe, 1992). Hence, it is necessary to locate areas for release of *R. antirrhini* that have not had flowering eliminated due to damage caused by *M. janthinus*. This has become difficult because *M. janthinus* has widely established and is spreading naturally, as well as being manually redistributed.

## Methods

The initial introduction of *M. janthinus* involved placement of the agent into propagation tents. Its redistribution to open field sites began in 1994. Adults of *R. antirrhini* were placed in the propagation tents in 1993, and open releases began in 1999. As little was known about the habitat requirements of *R. antirrhini*, efforts have been made to release *R. antirrhini* into temperate to mild habitats, with the hope of ensuring establishment. Both agents were subsequently redistributed across the province, and data describing host plant populations, biological control agent releases, and monitoring for weevil establishment, habitat preference and dispersal are being recorded.

Extensive field work has been and continues to be carried out to gather data about Dalmatian toadflax and its management. Historical data were incorporated into IAPP upon its launch (2005). Since then, data has been added as management activities and evaluations were carried out.

Site data in IAPP is displayed spatially using dots and polygons to represent sites where the biological control agents have been released. With a baseline of habitat information, additional environments can be tested for habitat preferences by over-laying the spacial information with habitat information, such as the BEC zone data.

**Figure 1.** Release site for *Mecinus janthinus* on Dalmatian toadflax, 2004, before control.

**Figure 2.** Release site for *Mecinus janthinus* on Dalmatian toadflax, 2006, after control.

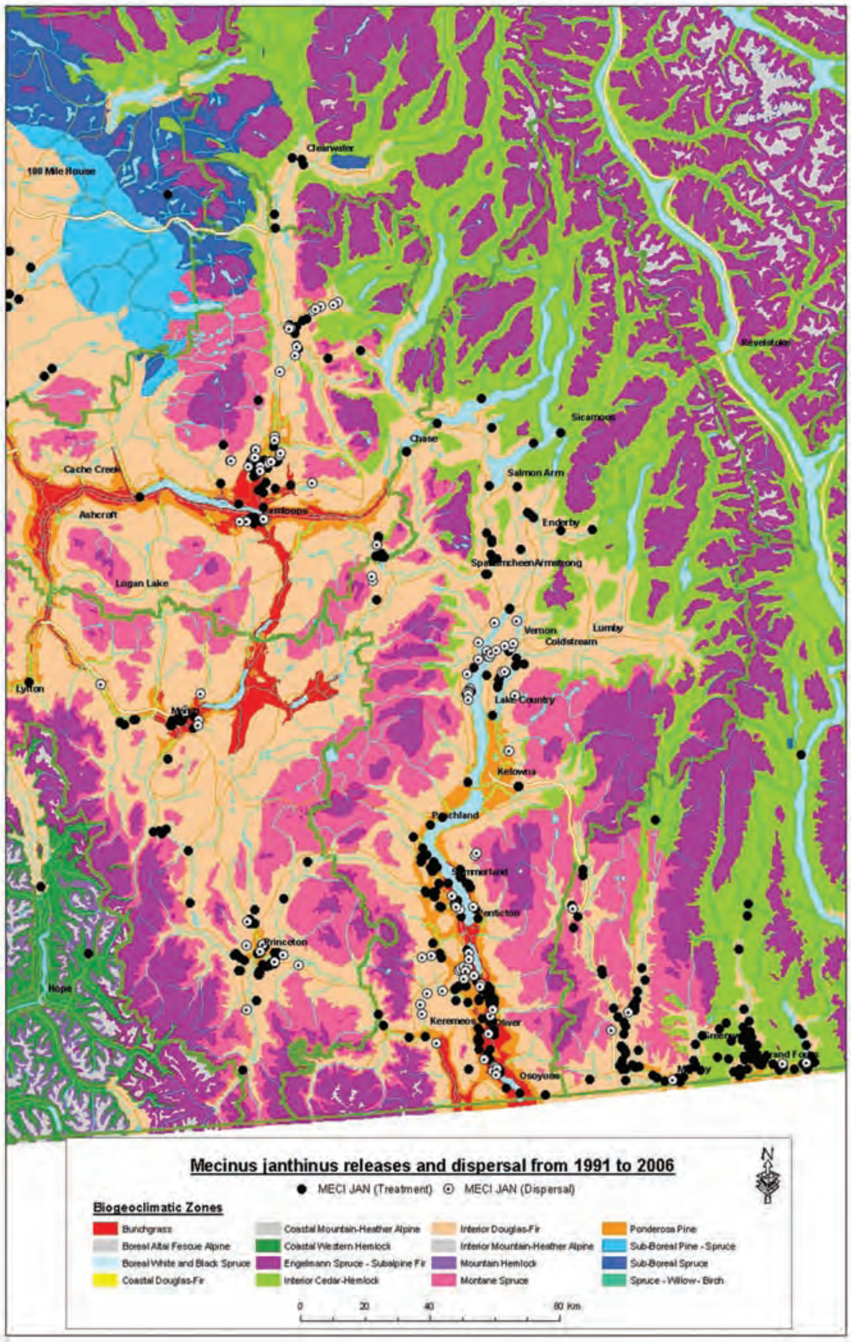


**Figure 3.** Release sites (black dots) between 1991 and 1999 and redistribution sites (open dots) to 2006 for *Mecinus janthinus* released onto Dalmatian toadflax in British Columbia.

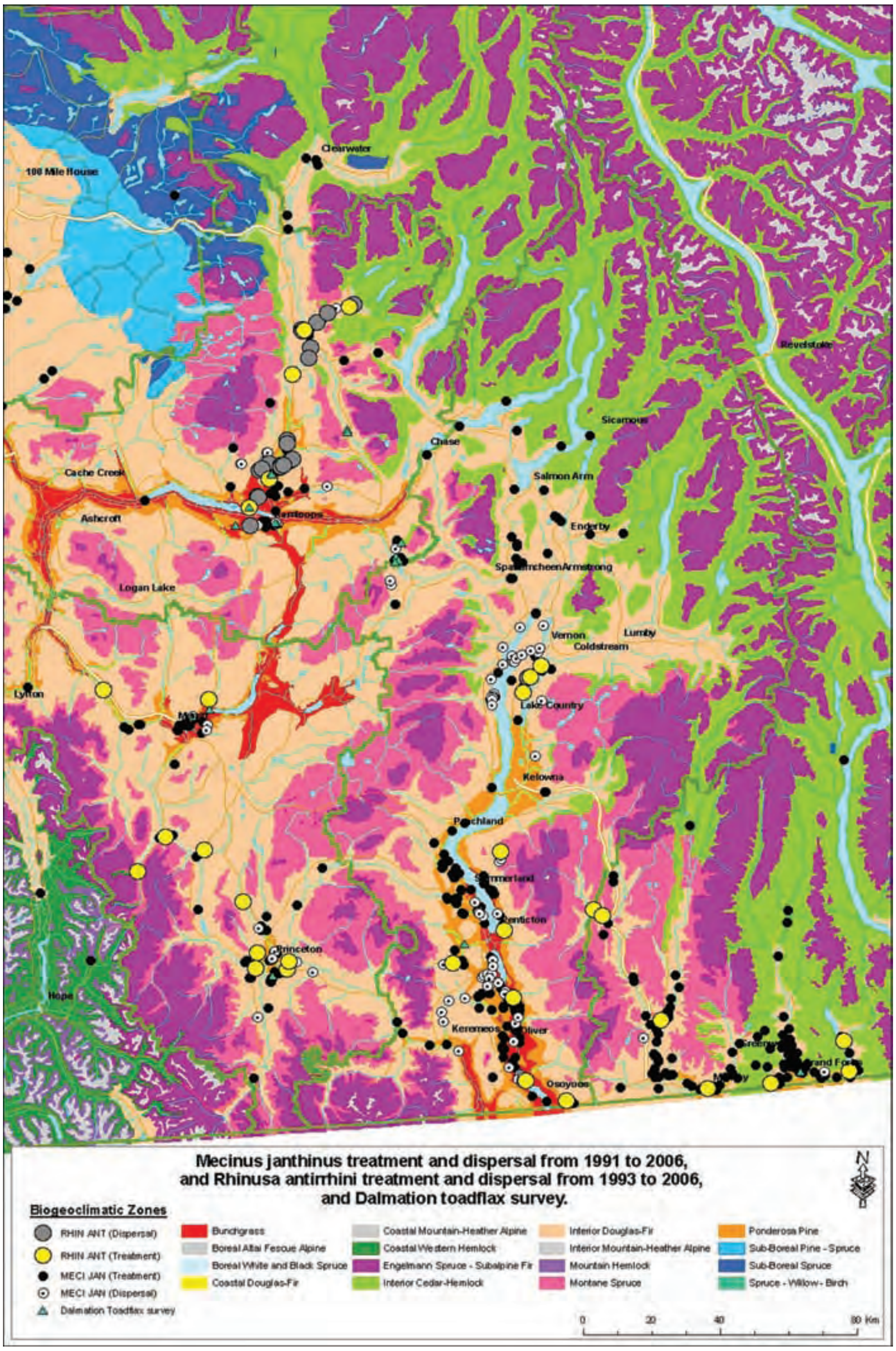


**Figure 4.** Release sites (yellow dots) between 1993 and 1999 and redistribution sites (grey dots) to 2006 for *Rhinusa antirrhini* on Dalmatian toadflax overlaid on the release and redistribution sites for *Mecinus janthinus* (Fig. 3).

IAPP has been used to select new redistribution sites, track dispersal of biological control agents and evaluate suitable habitats for establishment. Upon evaluation of IAPP data, sites have been selected that are free of (or have low populations of) *M. janthinus* for *R. antirrhini* redistribution.

## Results

The two weevils, *M. janthinus* and *R. antirrhini*, have been redistributed to 719 and 34 sites, respectively, by autumn 2006. In the field, *M. janthinus* has exceeded its predicted distribution in British Columbia. For example, a pair of *M. janthinus* weevils was found at Terrace 1 year after the agent's release at this location, and 2 years after the release evidence of characteristic stem-mining was observed. The northern city of Terrace is 54°30′N and approximately 280 km further north than the predicted 52°N limit for this insect. Terrace's climate is tempered by its proximity to the Pacific Ocean, and its climate is described as the BEC zone Coastal Western Hemlock, sub-montane, wet sub-maritime. It has a rather mild climate compared to the surrounding areas.

Another city, Williams Lake, 52°08′N, is at the northern edge of the predicted limit for *M. janthinus*. This interior city is influenced by the cold climate of the coastal mountain range and the open Cariboo Plateau. However, as it is located next to a large lake its climate is also tempered. It is described as the BEC zone Interior Douglas-fir, very dry, mild. A thriving *M. janthinus* population at Williams Lake has yielded thousands of weevils for redistribution. Additionally, *M. janthinus* has had a significant impact on its host plant at many of the release sites there.

Populations of *R. antirrhini* have been found to establish in all BEC zones in which this species has been released, including the BEC zones Bunchgrass, Ponderosa pine, Interior Douglas-fir, Interior cedar-hemlock and Montane spruce. The lowest and highest recorded elevations were 290 and 1205 m, respectively.

## Discussion

The process of redistribution and monitoring of biological control agents continues in the attempt to control Dalmatian toadflax. This objective is complicated by the number of different agencies and organizations conducting field work and the natural dispersal of the agents. The use of IAPP to record release and dispersal information is a recent development; thus, the data are just beginning to accumulate (Fig. 3), and preliminary results have been generated (Fig. 4).

When initial releases were monitored and the agents were found to have established, new, more extreme environments were tested, and dispersal information was recorded. The habitat requirements for the individual biological control agents then became more clear. When the goal of a biological control program is to have the invasive alien plant species under control by a long-term, self-sustaining system, it is critical to understand the habitat requirements and natural dispersal of the biological control agents.

Eventually, the compilation and evaluation of the data contained within the IAPP application should allow a comprehensive assessment of the complex of biological control agents for individual target plant species. The spread of Dalmatian toadflax in BC is likely to continue until the plant reaches its ecological limits. Using IAPP, comparison of the data describing this spread with the habitat-requirement data of its biological control agents will enable weed managers to determine whether sufficient, suitable agents exist in the province, where further redistribution of such agents is required and whether introduction of new agents should be pursued.

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# Monitoring of ground cover post release of *Aphthona nigriscutis* near Lander, Wyoming

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In 1990, *Aphthona nigriscutis* was released for the control of leafy spurge (*Euphorbia esula*) at 20 locations near Lander, WY. Establishment at several locations by 1993 prompted a post-release monitoring effort. Permanent transects were established at many locations. Eight sites are still usable, the rest being lost to development or herbicide use. At four of these sites, five 50-ft (15.25-m) transects radiate out from the initial point of release at approximately 70° intervals. Ground cover was measured at 5-ft (1.52-m) intervals along each transect by using a ten-pin point frame, recording the first contact with each pin. At the other four sites, there were eight radial transects, 100 ft (30.5 m) long, with data collected at 10-ft (3.04-m) intervals. The sites differ by slope, aspect, soil type and moisture. Data has been collected annually at the peak of vegetative production in late July or early August. Since 1993, leafy spurge has declined from 52% of the ground cover to 11% across all eight sites. Perennial grasses and forbs have increased, while bare ground has declined.

# Benefits to New Zealand's native flora from the successful biological control of mistflower (*Ageratina riparia*)

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A white smut fungus (*Entyloma ageratinae*) and a gall fly (*Procecidochares alani*) were released in New Zealand to suppress the invasive weed mistflower (*Ageratina riparia*). The impacts of the agents on the target weed and surrounding vegetation were monitored over 5 years. The fly was released 3 years after the fungus and spread much more slowly, so most of the impacts observed are attributable to the fungus alone. Nonetheless, the number of stem galls produced by the insect increased exponentially to reach damaging levels at release sites. Annual monitoring of study plots revealed that the mean percentage of leaves infected by the fungus reached 58%, and there was a significant decrease in the maximum height of mistflower plants. In heavy infestations, the mean percentage cover of mistflower declined from 81% to 1.5%. As the weed declined, the mean species richness and percentage cover of native plants increased to approach that of areas without mistflower. In contrast, there was no significant change in the species richness or percentage cover of exotic plants (excluding mistflower). There was a weak 'replacement weed effect' from the invasive African club-moss (*Selaginella kraussiana*), but mostly, the decline in mistflower benefited indigenous plants, including two rare endemic *Hebe* species.

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# Tracking population outbreaks: impact and quality of *Aphthona* flea beetles on leafy spurge at two spatial scales

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As classical biocontrol agents go through their first outbreak cycle, the impact, natural dispersal and quality of insects available for redistribution become important issues. *Aphthona lacertosa* populations were monitored at 21 sites for 6 years in Alberta, Canada. Impact and weed population changes were assessed initially at a release stake-scale (10-m circle) and then at the scale of the release patch. Adult beetles were sampled between three and four times per year, and stem counts and release patch perimeters were assessed annually using a global positioning system. Releases that occurred in 1997 resulted in 100% establishment in 1998 and visible damage (halos of dead spurge) at 81% of the release sites. First outbreak populations of beetles occurred in 2000, with maximum reductions in spurge density approaching 100% at some locations. Spurge populations recovered in the drought year of 2001. In subsequent years, many sites experienced a re-infestation with spurge seedlings, although beetles were still present. Beetle quality attributes, including sex ratios, size and egg loads, were measured at each site during population outbreaks. Sex ratios were even and relatively consistent, whereas beetle size and egg loads varied within the season, which may influence the spread and establishment of new sites.

# Are nutrients limiting the successful biological control of water hyacinth, *Eichhornia crassipes*, in South Africa?

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Water hyacinth remains a problem aquatic weed in South Africa and throughout the world due to eutrophic waterbodies. Previous work correlated water hyacinth growth with nitrogen and phosphorus in particular. Our field sampling tested the trade off between nutrient levels and biological control by country-wide monitoring of 14 water-hyacinth-infested sites. Water quality, insect population parameters and growth of water hyacinth plants was measured monthly for 24 months. High levels of inorganic nitrogen (7.5 mg/l) and phosphates (1.37 mg/l) caused a significant increase in petiole length, ramet production and biomass. Sites with limiting levels of these nutrients resulted in no or a reduced amount of growth. Higher levels of insect damage were recorded at eutrophic sites due to higher nitrogen content in the leaf tissue (5% N). Insect damage was still significant at nutrient limited sites where biological control has been successful. We conclude that eutrophication decreases the successful biological control of water hyacinth. Nutrients override the effects of temperature, and biological control has achieved more success on water bodies that are nutrient limited due to reduced plant growth.

# Spatial evaluation of weed infestation and bioagent efficacy: an evolution in monitoring technique

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Traditional invasive weed and insect biological control agent monitoring can be time consuming, costly and labour-intensive. Practices involving linear transect sampling provide relatively low return on a monitoring investment, particularly when trying to understand target plant and bioagent spread and establishment. Global positioning system and geographical information system (GIS) are tools that are rapidly replacing tape measures and topographic maps. Over the past decade, electronic data collection and analysis techniques have evolved along with the widespread availability of such technologies. Information is now gathered in a spatially relevant manner to identify key environmental attributes contributing to the efficacy of our biocontrol efforts. This presentation will contrast information typically obtained from traditional transect vegetation sampling methods against data collected and evaluated using GIS. Weed and insect population patterns that are readily apparent using a spatial approach, such as 'hotspots' and patchiness, edge effects and directional trends, are generally unseen using traditional data collection techniques. GIS analysis allows for an improved statistically sound method of analysing biocontrol efficacy. It also facilitates a much more predictive approach to biocontrol agent release by utilizing spatial modelling techniques. These concepts will be discussed using field-collected data on the biological control of knapweeds, leafy spurge and Dalmatian toadflax.

# Influence of release size on the establishment and impact of a biocontrol root weevil

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A strategic approach to classical weed biocontrol implementation is emerging through recent efforts to predict success in agent population establishment and persistence based on initial release size. However, there has been little formal investigation of whether release size can also predict agent efficacy. A root-boring weevil, *Mogulones cruciger*, which was introduced in Canada to control houndstongue (*Cynoglossum officinale*), was used to test this relationship. Numbers of 0, 100, 200, 300 or 400 weevils were field-released within discrete, isolated houndstongue patches (five replicates per treatment). Patches were monitored over 2 years for weevil establishment, host attack and changes in houndstongue populations. The weevil established successfully in all treatment patches, regardless of release size. Release size was positively correlated with weevil numbers and damage to host plants. In contrast, the different release sizes reduced houndstongue populations by the same amount and at the same rate relative to control patches during the 2-year study. Thus, all release sizes tested could predictably achieve patch-level control of houndstongue despite differences in the level of feeding. The measurable and predictable impact of the agent on weed populations in this system are mainly attributed to reliable agent establishment and rapid kill of host individuals.

# Development of *Mycoleptodiscus terrestris* as a biological control agent of *Hydrilla*

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In aquarium studies, *Mycoleptodiscus terrestris* has previously been shown to be effective in controlling the invasive aquatic weed, *Hydrilla verticillata*. The development of *M. terrestris* into a commercially viable control option requires methods to produce, formulate and disperse infective propagules. Our current research addresses these problems at the pilot plant scale. Microsclerotia of *M. terrestris* are produced in liquid culture fermentation, processed and dried for enhanced survival. Methods and handling parameters have been developed to produce uniform-sized particles, which are amenable to the drying process. The drying process has been optimized to provide stabile propagules with a long shelf-life. In addition, analysis of the chemical composition of the microsclerotia suggests that the membrane stabilizers, trehalose and mannitol, are important to its drying tolerance.

# Molecular characterization of *Striga* mycoherbicides '*Fusarium oxysporum* strains': evidence for a new forma specialis

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*Fusarium oxysporum* isolates (Foxy 2 and PSM197) are potential, highly host-specific mycoherbicides for the control of the parasitic weeds *Striga hermonthica* and *Striga asiatica*, which are major biotic constraints in cereal in semi-arid tropical Africa. The fungal isolates were cultivated on potato dextrose agar medium and characterized based on partial DNA sequence of the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene. The ITS sequence was obtained using the universal primers ITS1 and ITS4. Both isolates were identical in ITS sequence. The ITS sequence obtained was not identical to any ITS sequence deposited in GenBank. By sequencing a non-coding, non-genic region of the samples, it was possible to differentiate between the strains Foxy2 and PSM197. In addition, primers for the detection of both isolates were developed. These primers were able to amplify sequence stretches from both strains with high sensitivity and without false-positive reactions to other Ascomycetes tested. These findings are helpful in monitoring the establishment, spread and persistence of *Striga* mycoherbicides in the soil, for quality control of mycoherbicide products and for proving their environmental biosafety by being able to distinguish them from wild, crop-pathogenic strains. The unique ITS sequence of the two isolates provides strong evidence to consider these pathogens of *Striga* as a new forma specialis (f.sp. *strigae*) that will facilitate and encourage the introduction and acceptance by regulatory authorities and farmers of *Striga* mycoherbicides for practical field application.

# Prioritizing candidate biocontrol agents for garlic mustard based on their potential effect on weed demography

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To reduce the possibility for non-target effects, biological weed control programs should select and introduce the minimum number of host-specific natural enemies necessary to control an invasive non-indigenous plant. However, selection of the best agent or agent combination is no easy task and depends on the ability to forecast the anticipated impact of each herbivore species on host-plant demography. In a project on the biological control of garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara and Grande] in North America, we experimentally investigated the impact of candidate agents on survival and reproductive output of the target plant. Results were combined and fed into a demographic model to explore the potential impact of each agent at the plant population level. Using these a priori analyses, we propose potential release strategies for the candidate agents in North America.

# The accidentally introduced Canada thistle mite *Aceria anthocoptes* in the western USA: utilization of native *Cirsium* thistles?

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*Aceria anthocoptes* (Acari: Eriophyidae) is a Eurasian mite that feeds on leaves and stems of Canada thistle, *Cirsium arvense*. *A. anthocoptes* was introduced in North America and now appears to be widely established, at least in the northern USA, where Canada thistle is a widespread exotic weed. *A. anthocoptes* is one of two *Aceria* mites recorded from *C. arvense* in Europe; no eriophyid mites had previously been collected from Canada thistle in the United States. In 2005 and 2006, we studied the biology of *A. anthocoptes* at several Canada thistle sites in northern Colorado, USA. We also sampled populations of four native thistles (*Cirsium canescens*, *Cirsium scariosum*, *Cirsium scopulorum* and *Cirsium undulatum*) to detect mite populations. These four thistles occur in grassland, foothills and montane habitats in Colorado and often grow in close proximity to Canada thistle. We collected eriophyid mites from all native plants; mite densities were similar to, or even exceeded, densities from adjacent Canada thistle populations. For now, we are presuming that collected mites are *A. anthocoptes*, though taxonomists are confirming identifications. If specimens are determined to be *A. anthocoptes*, our information should preclude the use of this mite as a *C. arvense* biocontrol agent in the United States.

# Formulation of *Colletotrichum truncatum* into complex coacervate – biocontrol of scentless chamomile, *Matricaria perforata*

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*Colletotrichum truncatum* (schwein.) Andrus and W. D. Moore is a phytopathogenic fungus to scentless chamomile, *Matricaria perforate* Mérat, a noxious weed in western Canada. High virulence and host specificity of the fungus towards scentless chamomile allowed considering it as a potential candidate for weed biocontrol. Microencapsulation of *C. truncatum* conidia in a complex coacervate has been investigated. Complex coacervates have been widely used as a microencapsulation technique for oil-dispersible active ingredients in the pharmaceutical and food industries. Conidia of *C. truncatum* are hydrophilic and oil-indispersible; therefore, an initial formulation step of suspending them in a water/oil invert emulsion was required before encapsulating in a complex coacervate. To maximize percent conidial encapsulation, parameters such as wall materials, i.e. protein-polysaccharide, stirring speed, surfactants, and conidial suspension to oil ratios, were optimized. Weed control efficacy of the formulations was determined on scentless chamomile at the six to eight leaf stages both under greenhouse and field conditions. In addition, the synergistic effect between the *C. truncatum* formulation and the herbicide Sencor® was evaluated. In this paper, our new approach to formulate *C. truncatum* in a complex coacervate and the efficacy assay results are presented, and implications for controlling scentless chamomile are discussed.

# Efficacy of the seed feeding bruchid beetle, *Sulcobruchus subsuturalis*, in the biological control of *Caesalpinia decapetala* in South Africa

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The release of the seed beetle, *Sulcobruchus subsuturalis*, for biological control of *Caesalpinia decapetala* in South Africa has been ongoing since 1999. We assessed seed dispersal and the effects of *S. subsuturalis* on seed banks, seedling recruitment and seed viability within five sites. Seeds were not dispersed beyond 10 m of the parent plant. This partly reduces the chances of seeds reaching suitable microsites and partly facilitates high seed mortality near the parent plant given that high beetle densities ensure that many seeds are colonized. On average, no site contained more than 5 seeds/$m^2$ because the beetle attack destroys seeds. Therefore, the seed bank is very small, possibly not lasting a year. One seedling established per square metre on average within all sites, suggesting that *S. subsuturalis* may have no effect on the population dynamics of *C. decapetala* despite the great impact on seed viability; overall, 93.7% of predated seeds were not viable. As seed-feeding agents alone rarely reduce weed density, additional agents should be released to attain successful control of this weed.

# Field studies of the biology of the moth, *Bradyrrhoa gilveolla*, as a potential biocontrol agent for *Chondrilla juncea*

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The root-attacking moth, *Bradyrrhoa gilveolla*, has been released as a potential biocontrol agent of *Chondrilla juncea* in Argentina and Australia; however, both efforts failed. As part of our effort to establish this insect in North America, we have conducted an in-depth field study of its biology in northern Greece with the goal of making a more informed release in which we synchronize the phenologies of this insect and its host. Our study population is at 950 m altitude in the mountains of northern Greece, an area climatically matching our intended release area, the interior mountains of the state of Idaho (USA). The field population has a moderate incidence of disease and parasitism, but its population has still remained fairly high for the 3 years it has been studied. Besides obtaining a much more complete picture of its basic field biology, the most interesting discovery is that despite living in an area with a long, cold, snow-covered and extensive winter, this insect has no distinct over-wintering stage or synchronized period of emergence of adults during the summer. Another interesting aspect of this study is the way that the larvae react and protect themselves from severe and cold winters.

# Release of additional strains of the rust, *Phragmidium violaceum*, to enhance blackberry biocontrol in Australia

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Additional strains of the leaf-rust fungus, *Phragmidium violaceum*, were approved in early 2004 for release in Australia to improve the prospects of biologically controlling European blackberry (*Rubus fruticosus* agg.) across the wide range of taxa and genotypes that exist. In tests before release, the additional strains were found to be pathogenic on all *Rubus* genotypes tested except the clones of *Rubus laciniatus*. Only one strain (G18-TG-00-4-1) was capable of infecting clones of this species. Results from host-specificity tests concurred with previous findings that *P. violaceum* does not pose a threat to commercial blackberry cultivars and Australian native *Rubus* species. As the additional strains cannot be distinguished from existing populations of *P. violaceum* in Australia, several avenues have been pursued to develop a simple and robust molecular diagnostic tool to monitor their establishment after release. The challenges in developing such a tool will be presented. The strains are currently being released on a national scale in partnership with land holders.

# Impact of the bridal creeper rust fungus, *Puccinia myrsiphylli*

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The rust fungus, *Puccinia myrsiphylli*, released in 2000 for the biological control of bridal creeper (*Asparagus asparagoides*) in Australia, is the most widespread and effective agent against this environmental weed of national significance. We used a glasshouse experiment to determine how different levels of artificial defoliation and rust fungus infection affect bridal creeper growth parameters, such as below-ground biomass and regrowth. Every fortnight for a total of 20 weeks, plants with a standardized number of tubers were manually defoliated (by 25%, 50%, 75% and 100%) or sprayed with a suspension of rust spores in water (104 and 105 spores per millilitre). Tuber number, relative growth rate and rhizome length of plants sprayed with the highest density of rust spores were similar to that of the 75% defoliated treatment. Most of the plants sprayed with the highest spore density and a few from the 100% defoliated treatment never regrew after the last treatment application. We also conducted a fungicide-exclusion field experiment at Camden, NSW, using standardized bridal creeper in pots, to determine the impact of natural rust fungus infection. Similar reductions in tuber number, rhizome length and regrowth to that observed in the glasshouse experiment were recorded in the field.

# Overview of the biological control of the invasive plant *Chromolaena odorata* (Asteraceae) in the Old World

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*Chromolaena odorata* (Asteraceae) is of Neotropical origin, introduced as an ornamental plant to the Calcutta Botanical Gardens, India, in 1836. This plant is highly allelopathic. It occupies vacant lands, pastures, disturbed forests, game reserves, roadsides and orchards. In the dry season, the tops dry up and become a fire hazard. For the biological control of this weed, several natural enemies were screened, and of these, the arctiid moth, *Pareuchaetes pseudoinsulata*, and the tephritid gallfly, *Cecidochares connexa*, have proven effective. *P. pseudoinsulata* has been established in Ghana, India, Malaysia, Sri Lanka, Indonesia, Philippines, Guam, Saipan, Rota, Tinian, Pohnpei, Chuuk, Kosrae, Yap and Papua New Guinea. *C. connexa* has been established in Indonesia, East Timor, Philippines, Guam, Rota Saipan, Palau Chuuk, Yap, Pohnpei, Kosrae and Papua New Guinea. In 1922, the International Organization for Biological Control formally approved the establishment of the Global Chromolaena Working Group. Seven international workshops have been conducted, one in each of Bangkok, Thailand (1988); Bogor, Indonesia (1991); Abidjan, Ivory Coast (1993); Bangalore, India (1996); Durban, South Africa (2000); Cairns, Australia (2003) and Pingtung, Taiwan (2006). Six proceedings and 16 newsletters were published.

# *Trichopria columbiana* – a pupal parasite of the *Hydrellia* spp. introduced for the management of hydrilla

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Parasitism by *Trichopria columbiana* has been suggested as a possible factor limiting population size and associated damage by *Hydrellia pakistanae* and *Hydrellia balciunasi* as biocontrol agents for hydrilla management. Parasitism impacts to emergence and population size were examined and hydrilla stems collected to determine immature numbers and leaf damage. Pupae were also collected and held individually to quantify actual parasitism. Limited impact is assumed, as *Hydrellia* spp. populations increased to almost 6000 immatures per kilogram, with leaf damage approaching 36% despite parasitism levels of almost 30% at the end of the growing season with even higher fly population levels found in following years. Host selection behaviour of *T. columbiana* was also quantified with four behavioural categories identified: grooming/resting, stem examination, searching and ovipositioning. Wasps spent the greatest percentage of time searching for prey and the least examining stems. Ovipositioning time increased in wasps ready for egg deposition only. Choice experiments addressing ovipositing preference of *T. columbiana* to specific life stages and associated wasp survivorship found that the intermediate pupal stage was both preferred for ovipositing and exhibited the highest percent survivorship.

# What is responsible for the low establishment of the bridal creeper leaf beetle in Australia?

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The leaf beetle *Crioceris* sp. was released in 2002 for the biological control of bridal creeper (*Asparagus asparagoides*), one of southern Australia's worst environmental weeds. The leaf beetle feeds on young bridal creeper shoots and emerges at the beginning of the growing season before other biological control agents (rust fungus *Puccinia myrsiphylli* and leafhopper *Zygina* sp.) become active. However, establishment and population build-up of the leaf beetle has so far been disappointing because it only established at three of 16 sites where it was released in 2002 and 2003. Glasshouse and field experiments were performed to determine possible causes for low establishment rates. Results confirmed that our rearing colony is producing highly fecund females. Temperature, however, significantly affected the number of eggs laid, time to hatch and number of larvae produced. The field experiments indicated that predation may limit the survival and establishment of the leaf beetle in the field, but additional experiments including more replicates are required to confirm observations. We recommend releases of large numbers of beetles to compensate for any predation or parasitism that may occur. Alternatively, exclusion cages could be used at the time of release to enhance initial increases in leaf beetle populations.

# Introduction, specificity and establishment of *Tetranychus lintearius* for biological control of gorse in Chile

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During the late 1990s, the gorse spider mite, *Tetranychus lintearius*, was introduced into Chile for the biological control of *Ulex europaeus* (gorse). To obtain approval for release, the mite was subjected to host-specificity testing. The target and 11 test plants species were exposed to the mite in no-choice and choice tests. Mite development, oviposition and frass deposition were assessed. Mite development through a next generation occurred on gorse only. Gorse was highly preferred compared to the test plant species in all the assays ($P < 0.001$). Spider mite frass proved to be a reliable parameter, indicating feeding (acceptability) to evaluate specificity. High specificity shown for the mite lead to its release between 1997 and 2000 at sites between the 37° and 42°S, and since then, it has established at 90% of the sites. After 9 years, the mite populations increased more than 1000-fold at many sites and spread up to 20 km in 3 years. Mite colonization, dispersal and plant damage were stronger in dry relative to humid areas. This is the first time that a spider mite has been introduced, subjected to host specificity tests and established in Chile and in South America.

# Were ineffective agents selected for the biological control of skeletonweed in North America? A post-release analysis

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Three agents were released for the biological control of rush skeletonweed, *Chondrilla juncea* L., in North America between 1975 and 1977. Although all three species are widely established, weed densities are increasing. Aside from anecdotal reports, there is little quantitative information on the efficacy of the biological control agents or factors that limit population size or impact. Thus, the question of whether ineffective agents were selected or effective agents are hampered by unpredicted environmental factors remains unanswered. We studied attack rates for the rush skeletonweed gall mite, *Aceria chondrillae* Canestrini (Acari: Eriophyidae), and the rush skeletonweed rust, *Puccinia chondrillina* Bubak and Sydenham. We found that, despite the potential for rapid population growth of both agents, winter mortality for the gall mite was higher than 90% in two consecutive years, and the rust was attacked by the mycoparasite *Eudarluca caricis* (Fr.) Eriksson. In addition, there was host-plant resistance to at least the rust. Under favourable environmental conditions (mild winter climates and absence of the mycoparasite), both agents impair rush skeletonweed in North America. We argue that the gall mite and the rust fungus are effective biocontrol agents but that their efficacy is hampered by the heterogeneity of habitats invaded and the diversity of rush skeletonweed genotypes introduced in North America.

# Confirming host-specificity predictions for *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*

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An underlying assumption of weed biological control asserts that laboratory-based host-specificity testing accurately predicts the realized host range of herbivores after release. We tested this assumption for the Australian weevil, *Oxyops vitiosa*, which was introduced into Florida (USA) for the biological control of *Melaleuca quinquenervia*. A series of common garden experiments were conducted to determine if *O. vitiosa* would exploit species predicted to be non- or suboptimal hosts in host-range tests. Adult immigration into replicated common gardens was influenced by species, with >90% of individuals located on *Melaleuca* hosts. While adults alighted on 78% of the test plants, oviposition was restricted to *Melaleuca* species and the exotic *Psidium guajava*. All stages of *O. vitiosa* larvae were observed on the three *Melaleuca* species while only first instars occurred on *P. guajava*. Mean larval densities were greatest for *M. quinquenervia*, which represented 92% of all larvae observed. The residency time for marked weevils placed on test species was greatest for *Melaleuca* congeners, which also recruited 98% of all recovered weevils. Felling the *M. quinquenervia* stand that surrounded a common garden resulted in high levels of immigration within the study plots, but feeding and oviposition on non-target plants were nonexistent. These results support the premise that risk assessments based on physiological host ranges are conservative when compared to realized ecological host ranges.

# Biological control of the ivy gourd, *Coccinia grandis* (*Cucurbitaceae*), in the Mariana Islands

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The invasive plant, ivy gourd, *Coccinia grandis*, is of African origin and was introduced to Guam and Saipan in the 1980s. It has occupied more than 200 ha in different parts of Guam and about 2000 ha of Saipan. A biocontrol program has been initiated in Guam and Saipan by introducing the natural enemies *Melittia oedipus* Oberthor (Lepidoptera: Sessidae), *Acythopeus burkhartorum* (Coleoptera: Curculionidae) and *Acythopeus cocciniae* (Coleoptera: Curculionidae). The Animal and Plant Health Inspection Service of the US Department of Agriculture has accepted the list of species used for host-specificity tests in Hawaii, and it was decided that the endemic species, *Zehnaria guamensis* (Cucurbitaceae), be tested in Guam. Host-specificity tests of these biocontrol agents were conducted at the quarantine facility in Guam. In May 2003, *A. cocciniae* was field-released in Guam and Saipan. It has established in both islands and caused defoliation of *C. grandis* by the larval mining of the leaves. *Acythopeus burkhortorum* was field-released in Guam in October 2004 and on Saipan in February 2005. Its field establishment on Guam has been confirmed, and its establishment in Saipan is yet to be verified. Host-specificity tests on *M. oedipus* are being carried at the University of Guam Quarantine facility.

# Quantifying the impact of biological control: what have we learned from the bridal creeper-rust fungus system?

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Evaluating the post-release impact of biological control programs is often neglected due to inadequate resources, time constraints or the perception that any success should be obvious. A quantitative review of the Australian weed biological control literature revealed that the majority of programs have not been adequately evaluated. While many studies have only focussed on assessing agent establishment and damage caused to individual plants, few have quantified impact of agents on weed populations and consequences for associated plant communities. The advantages and disadvantages of various approaches to measure impact of agents will be discussed using as a case study the rust fungus, *Puccinia myrsiphylli*, released for the biological control of bridal creeper (*Asparagus asparagoides*). This agent has been extensively evaluated using glasshouse and field-manipulative experiments as well as pre- and post-release monitoring of impact on field populations. General guidelines for evaluating impact of agents will be presented.

# From invasive to fixed-in-place: the transformation of *Melaleuca quinquenervia* in Florida

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*Melaleuca quinquenervia* (melaleuca) once spread unimpeded across the south Florida landscape, infesting 0.61 million ha at its height. The complete lack of top-down regulation of its growth and reproduction resulted in its rapid spread into pine flatwoods, cypress domes, sawgrass prairies and hardwood hammocks. The first biological agent, *Oxyops vitiosa*, was introduced in 1997 and the second, *Borelioglycaspis melaleucae*, in 2002. These natural enemies, especially *O. vitiosa*, have transformed both the habit and reproductive capacity of *Melaleuca*. Plants attacked by *O. vitiosa* grow slower (9.1 vs 96.1 cm year$^{-1}$); produce many more tips (4.2 vs 2.8 tips cm height$^{-1}$) resulting in a shorter, bushier plant, produce fewer seed capsules (0.006 vs 0.343 capsule clusters centimetre per tree height), resulting in 97.5% less seed per tree. Existing tree densities have declined 35.4% since 2002 when not protected from these natural enemies, while protected areas increased by 9.4%. In another study, *Melaleuca* was able to recruit only 2.4% of its previous density of seedlings/saplings. It is now clear that the capacity of *Melaleuca* to invade and dominate new habitats has been severely constrained by biological control.

# Long-term field evaluation of *Mecinus janthinus* releases against Dalmatian toadflax in Montana (USA)

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The toadflax stem mining weevil, *Mecinus janthinus*, was first released in the United States, in Montana, in 1996. This agent has now become established to varying degrees on subsequent releases made throughout the state. Multiple releases of *M. janthinus* have presented researchers with a unique opportunity to evaluate the efficacy of this agent in diverse habitats and under a variety of environmental conditions. The results presented in this paper summarize findings from long-term field data, illustrating not only the impact of *M. janthinus* on the target weed, Dalmatian toadflax, but also on correlated plant community dynamics. These results additionally provide a valuable means to compare and contrast the biotic response and control efficacy of this agent at both a regional and sub-continental scale.

# Population dynamics and long-term effects of *Galerucella* spp. on purple loosestrife, *Lythrum salicaria*, and non-target native plant communities in Minnesota

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An 11-year field study (1995–2006) assessed the effects of *Galerucella calmariensis* and *Galerucella pusilla* (Coleoptera: Chrysomelidae) on purple loosestrife, *Lythrum salicaria*, and non-target native plant communities in Minnesota. *Galerucella* spp. populations initially peaked between 3 and 5 years after establishment. At all sites, purple loosestrife density declined (up to 90%) in response to an increase in *Galerucella* spp. *Galerucella* spp. appear to have a strong numerical response to purple loosestrife density, which led to multiple 'boom and bust' cycles occurring on many of the sites during the 11-year period. Declines in *Galerucella* spp. typically allowed purple loosestrife populations to rebound. Generally, *Galerucella* spp. populations rebounded as loosestrife abundance increased. The number and amplitude of the boom and bust cycles appears to be related, in part, to the density of the initial purple loosestrife infestation. Sites where purple loosestrife approached 100% cover tended to cycle more frequently than sites with higher plant diversity and abundance. It appears that, in more diverse sites, increased plant competition prevented purple loosestrife from attaining pre-release densities. As purple loosestrife populations declined, plant species richness and/or abundance increased within release sites. We will discuss these results in context of overall success of the program.

# Midges and wasps gain tarsus hold – successful release strategies for two *Hieracium* biocontrol agents

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Hieracium gall midges (*Cecidomyiidae*: *Macrolabis pilosellae*) and gall wasps (Cynipidae: *Aulacidea subterminalis*) have been released in New Zealand to combat invasive *Hieracium* species (*Hieracium pilosellae, Hieracium praealtum, Hieracium caespitosum* and *Hieracium aurantiacum*). Gall wasps were first released in 1999 and gall midges in 2002. A strategy of releasing small numbers of individuals at many dispersed sites was adopted to overcome site specific factors, which may have precluded insect establishment. Wasp releases comprised either 100 newly emerged adults or 100 over wintered galls containing ready to emerge adults. Midge releases comprised 20–40 galled plants containing larvae and pupae, planted at each site. To date, 99 wasp and 136 midge releases have been made, with proven establishment at 32% and 92% of sites, respectively. In many instances, failed establishment or establishment and subsequent failure was attributed not only to drought but also to changes in land management, e.g. cultivation and spraying. Wasp gall densities were measured at up to 122 chambers per square metre six seasons post release. Midge galled plants were measured at up to 1.2 per square metre two seasons post release. Long-term biocontrol agent and vegetation monitoring has been established to quantify impact of wasps and midges on hawkweeds and other exotic and native plant species.

# Are seedfeeding insects adequately controlling yellow starthistle (*Centaurea soltitialis*) in the western USA?

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Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is an exotic plant infesting 7.5 million ha of land in the western USA. Six seedhead-feeding insects were released against this plant between 1984 and 1992. We conducted insect exclosure experiments at four yellow starthistle field sites in the Hell's Canyon ecosystem (Idaho) to determine whether insects solely targeting seedheads can adequately control yellow starthistle or whether the release of additional agents targeting different plant parts is warranted. We compared several plant response variables between plots sprayed with pyrethroid and imidacloprid insecticides and plots sprayed with equal amounts of water over the course of two consecutive field seasons. More than 80% of seedheads in control plots were attacked in 2005; more than 93% were attacked in 2006. Seed production in insecticide-treated seedheads was reduced by 27.1% and 58.5% in 2005 and 2006, respectively. However, yellow starthistle plant density increased from 2005 to 2006. Despite the continued high abundance and attack rates of seedhead feeding insects, it does not appear that the soil seed bank of yellow starthistle is sufficiently impaired to cause reductions in plant populations. Thus, the planned release of additional agents targeting roots and stems may greatly benefit the biological control program against yellow starthistle.

# Impact of the rust fungus *Uromycladium tepperianum* on the invasive tree, *Acacia saligna*, in South Africa: 15 years of monitoring

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In the early 1980s, *Acacia saligna* was rated as the invasive alien weed posing the greatest threat to the Cape Floristic region. The rust fungus, *Uromycladium tepperianum*, was introduced into South Africa from Australia in 1987 and established throughout the range of the weed. Five populations of the weed were monitored annually from 1991 to 2005, and the number of trees recorded in each of four permanent transects at each site. The tree density declined by between 87% and 98% from 1991 to 2005 at these sites. The average annual mortality rate (±SE) of infected trees dying at the sites was 18% (±2%). Trees were destructively sampled once at 19 sites during March to May of 2004 and 2005. The canopy mass at these sites was 12–90% lower than data published before the introduction of *U. tepperianum*. The average age of trees in these sites was between 2.2 and 6.1 years, despite the last major disturbance being at least 10 to 20 years previous at 11 of the sites. Pod and seed production was determined and seed fall calculated at four sites in 2004 and compared to data gathered in 1989. The average seed fall in 2004 was reduced by 89% compared to that recorded earlier. It is concluded that *U. tepperianum* continues to be a highly effective biocontrol agent against *A. saligna* in South Africa.

# Success at what price? Establishment, spread and impact of *Pareuchaetes insulata* on *Chromolaena odorata* in South Africa

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The defoliator, *Pareuchaetes insulata* (Lepidoptera: Arctiidae), was the first agent established on *Chromolaena odorata* (Asteraceae) in South Africa. Following the methods used to establish *Pareuchaetes pseudoinsulata* elsewhere, *P. insulata* from Florida was mass-reared and released at 17 sites in KwaZulu-Natal province from 2001 to 2003. Establishment was achieved at only one site, in the coastal town of Umkomaas, which received 380,000 larvae over 21 months, and even here, the insects virtually disappeared for 15 months. Because the rearing and release methods used should have overcome disease and Allee effects and climatic modelling indicated reasonable matching, the only obvious remaining reason for apparent non-establishment was poor matching with plant biotype. Therefore, Jamaican and Cuban populations were released at several sites from 2003 to 2005. Although initial patterns of increase and spread of these populations were better than with the Florida population, long-term success was poor. The Umkomaas population underwent a massive increase in 2005 and, by mid-2006, had defoliated large areas of chromolaena within a 4-km radius of the release site, causing death or decreased competitiveness of plants. Spread was evident 25 km along the coast and 10 km inland. Laboratory trials indicated that larval defoliation substantially decreased plant growth. Before this dramatic success, the programme drew criticism for its high cost and poor results. We discuss issues surrounding the appropriate termination point in attempting to establish an agent.

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## Theme 9:

# Management Specifics, Integration, Restoration and Implementation

## Session Chair: John Hoffmann

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Keynote Presenter

# Integration of biological control into weed management strategies

**J.M. DiTomaso[1]**

*Summary*

In addition to biological control, many other management techniques including mechanical, cultural and chemical options can be used to control invasive plants. Integration of these strategies with biological control can, in some cases, provide more effective long-term management of a particular weed than the use of a single control option. Herbicides do not generally impact insect populations or pathogens and therefore can be used without compromising the effectiveness of most biological control agents (BCAs). In some cases, particularly with aquatic weeds, the combination of sublethal herbicide concentrations and BCAs can act synergistically. Other studies have used integrated management systems that combine BCAs and prescribed burning. Although burning in spring or summer can kill exposed BCAs, both insects and pathogens are mobile organisms and have the opportunity to readily recolonize the treated site. In other situations, timely burning can increase available nitrogen or remove the soil litter layer, thus benefiting the population growth of BCAs by increasing access to the soil surface or improving the nutrient levels in the target plant. Biological control in concert with competitive, desirable non-target plants can also improve the control of some invasive plant species and prevent subsequent establishment of the weed. Although there are some studies that have demonstrated the benefits of incorporating biological control strategies with other management options, examples of this approach are few. However, with an increased research effort in this area, the potential for successfully using BCAs in an integrated weed management programme is very promising.

***Keywords***: IPM, invasive plant, biological control, integrated management.

## Introduction

The goal of any weed management plan should be not only to control the noxious plant but also improve the degraded community, enhance the utility of that ecosystem, and prevent reinvasion or invasion by other noxious weed species. To accomplish this often requires a long-term integrated management plan. Development of a management programme and selection of the proper tool(s) depends on many factors, including the specific weed species requiring management, its associated vegetative community, initial infestation density, effectiveness of the control techniques, time necessary to achieve control, environmental considerations, chemical use restrictions, topography, climatic conditions and relative cost of the control techniques (DiTomaso *et al.*, 2006c). A number of considerations can influence the choice of options, most important being the primary land-use objective, which can include forage production, preservation of native or endangered plant species, wildlife habitat development, water management and recreational land maintenance. A successful long-term management programme often includes combinations of mechanical, cultural, biological and chemical control techniques (DiTomaso *et al.*, 2006c).

Probably the least studied integrated strategy involves the combination of biological control with other management options. This is due in part to the complexity of such a system compared to other control options, the limited number of weedy species with well-established biological control agents (BCAs) and the interdisciplinary nature of such studies. However, the opportunity to integrate BCAs, whether insects or pathogens, into long-term integrated weed manage-

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ment strategies is numerous and have great potential for success.

## Integrated Approaches

In some cases, a combination of control options may be necessary to facilitate establishment of desirable vegetation or to prevent catastrophic wildfires. For example, in some locations in the western United States, the saltcedar leaf beetle (*Diorhabda elongata* Brullé) has proven to be a very effective defoliator of saltcedar (*Tamarix* spp.). Four to five years of continuous defoliation has begun to result in mortality of saltcedar (Milbrath *et al.*, 2003). Saltcedar in some of these areas can occupy large expanses and the resultant dried vegetative biomass will inevitably increase the fuel load that can potentially lead to large-scale wildfires. As a result, other control options, including prescribed burning and mechanical removal, are being considered as a subsequent option following the success of the insect agent. Although this is an example of an integrated management strategy, saltcedar control is primarily restricted to the use of the BCA. In contrast, most examples of integrated weed management using biological control rely on additional management tools to additively or synergistically contribute to the control of the weed. This review will describe a variety of integrated strategies that have been, or potentially may be, used successfully. Of the integrated approaches combined with biological control, most also involve herbicides, prescribed burning, and/or the use of competitive desirable vegetation.

### Herbicide and biological control agents

**Terrestrial invasive plants:** Many researchers have evaluated the direct impact of herbicides on weed BCAs (Table 1). In most cases, herbicides do not cause direct damage to insects, and thus can have excellent potential in integrated strategies with biological control organisms.

In some cases, however, herbicides can temporarily disrupt the establishment of a weed biological control programme. In leafy spurge (*Euphorbia esula* L.) stands treated in autumn with 2,4-D and picloram, *Aphthona* spp. were shown to be temporarily less abundant compared to untreated plots, with no significant long-term benefit to weed control (Larson *et al.*, 2007). The authors felt that the flea beetles may have abandoned the herbicide-treated patches for greater resources available in untreated plots. They concluded that there was little advantage to combining herbicides with biological control in areas where biological control is already considered successful.

However, in a yellow starthistle (*Centaurea solstitialis* L.)-infested area in California, the attack rates of the hairy weevil (*Eustenopus villosus* Boheman) and the false peacock fly (*Chaetorellia succinea* Costa) did not differ in the year after a clopyralid treatment (Pitcairn *et al.*, 2000). At this site, the insects did not avoid the treated areas, even though yellow starthistle plant density was considerably lower than the untreated adjacent sites. Lym and Carlson (1994) noted that the combination of herbicides and biological control was most effective for leafy spurge control if between 15% and 25% of the area was left untreated to sustain insect populations.

One of the key aspects in the proper use of herbicides in combination with BCAs is the timing of the chemical application. Although insect biological control and herbicides represent a very promising integrated management approach for purple loosestrife (*Lythrum salicaria* L.) in North America, applications of the herbicide glyphosate too early in the season can destroy the food source for the leaf beetle *Galerucella calmariensis* L. (Lindgren *et al.*, 1999). This is of particular concern where the beetles are well established. A late-season application of the herbicide, when plants are in bloom, is more compatible with *G. calmariensis*

**Table 1.** List of biological control insects, target weed species and herbicides that can be applied without damage to the insect.

| Insect biocontrol agent | Target weed species | Herbicide | Reference |
|---|---|---|---|
| *Rhinocyllus conicus* Froehlich | *Carduus* spp. | 2,4-D | Trumble and Kok, 1980 |
| *Ceuthorhynchidius horridus* Panzer | *Carduus* spp. | 2,4-D | Trumble and Kok, 1980 |
| *Sphenoptera jugoslavica* Obenberger | *Centaurea diffusa* Lam. | Picloram, Clopyralid | Wilson *et al.*, 2004 |
| *Cyphocleonus achates* Fahraeus | *Centaurea maculosa* Lam. | Picloram | Jacobs *et al.*, 2000 |
| *Urophora affinis* Frfld. and *U. quadrifasciata* Meig. | *Centaurea maculosa* | 2,4-D, Picloram | McCaffrey and Callihan, 1988; Story *et al.*, 1988 |
| *Eustenopus villosus* Boheman and *Chaetorellia succinea* Costa | *Centaurea solstitialis* L. | Clopyralid | DiTomaso *et al.* 2006 |
| *Hyles euphorbiae* L. | *Euphorbia esula* L. | 2,4-D, Picloram | Rees and Fay, 1989 |
| *Spurgia esulae* Gagne | *Euphorbia esula* | 2,4-D, Picloram | Lym and Carlson, 1994 |
| *Galerucella calmariensis* L. | *Lythrum salicaria* L. | Glyphosate, Triclopyr | Lindgren *et al.*, 1999 |

than an early season application because most adults will have already entered winter diapause. In this situation, *G. calmariensis* would potentially control young purple loosestrife seedlings in the following season (Lindgren *et al.*, 1999).

Autumn applications of 2,4-D and clopyralid were not compatible with two root-feeding insects, *Cyphocleonus achates* Fahraeus and *Agapeta zoegana* L., for the control of spotted knapweed (*Centaurea maculosa* Lam.), whereas spring treatments were compatible (Story and Stougaard, 2006). This was due to the low level of survival of the larvae following autumn application timing. By comparison, spring application of 2,4-D plus picloram for leafy spurge control was detrimental to *Aphthona* spp. establishment, as it eliminated the adult food source. However, autumn applications did not negatively affect establishment or reproduction of the flea beetles, and when used in combination, leafy spurge control was better and more economical than either method used alone (Lym *et al.*, 1996; Lym, 1998; Lym and Nelson, 2002). In the year after the herbicide treatment, leafy spurge stem density decreased by 85% to 95% when applications were made to plants infested with the BCAs (Lym *et al.*, 1996; Lym and Nelson, 2002). In contrast, when only the insects were present, it took 3 years longer to reduce the infestation to the same level achieved in 1 year with the integrated strategy. Once leafy spurge density was reduced, the *Aphthona* flea beetles maintained acceptable control for at least an additional 7 years (Lym and Nelson, 2002). This resulted in a threefold to fivefold cost savings to land managers who typically reapply herbicides annually for a number of years. Lym (1998) concluded that the benefit of this combination was due to the decrease in seed production through the activity of the insects and the inhibition in vegetative spread by creeping roots through the action of the herbicide. The response was even better when *Aphthona* spp. populations were already established at the time of the herbicide treatment (Lym and Nelson, 2002).

Another study integrated the root-feeding beetle *Sphenoptera jugoslavica* Obenberger with low rates of picloram or clopyralid for the control of diffuse knapweed (*Centaurea diffusa* Lam.). The results indicated that, compared to other months, a June herbicide application in Colorado (USA) was the most compatible with the insect and improved control compared to either technique used alone (Wilson *et al.*, 2004). The June treatment timing mimicked a midsummer arrest of diffuse knapweed growth that is normally triggered by late-summer drought. Insect damage is greater in drought-stressed plants than in other periods of the season when plants have adequate moisture. Herbicides applied in June increased infestation of diffuse knapweed plants by the root feeder by 25% the year after treatment compared to untreated areas. This response was suggested to be due to the herbicide-induced arrest in summer growth (Wilson *et al.*, 2004).

Pitcairn *et al.* (2000) hypothesized that combining clopyralid application with insect BCAs could provide more effective long-term control of yellow starthistle. While an initial clopyralid application would reduce plant density and new recruitment into the seed bank, subsequent activity of the BCA on seed production would slow the rate of reinfestation. In a field study to test this hypothesis, they found that 1 year following a clopyralid treatment, there were no significant differences in attack rates between the treated and untreated populations, despite the low density of yellow starthistle in the treated area (Pitcairn *et al.*, 2000). In the year after treatment, they also found that BCAs suppressed seed production by 76% compared to controls. If successful, the combination of the two control methods would reduce the need for continuous herbicide treatments.

**Aquatic invasive plants:** A number of studies have also demonstrated the value or potential value of using biological control in an integrated approach with herbicides in aquatic systems. For example, the efficacy of two biological control weevils, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache, was compared for control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms, in both a managed aquatic system, where the herbicide 2,4-D was routinely applied as a maintenance treatment, and an unmanaged site with no herbicide treatment. The maintenance treatment did not eliminate water hyacinth. Rather, it resulted in lower insect populations and fewer but healthier, more vigorous plants with higher nutritional quality (Center *et al.*, 1999). Despite the lower insect populations over time, the weevils benefited from the herbicide treatment compared to the untreated area. Intraspecific competition in the unmanaged site led to smaller, more stressed individual plants that were less suitable for weevil populations. The larger plants of the managed areas enhanced weevil reproduction and, in the long-term, provided more sustainable control of water hyacinth. The authors concluded that such an integrated approach can exploit the benefits of both methods while minimizing the negative aspects of each (Center *et al.*, 1999).

Combinations of sublethal herbicide rates and pathogens have been used synergistically to increase the susceptibility of the target species, while reducing the damage to non-target plants. This approach has been used for the control of Eurasian watermilfoil *Myriophyllum spicatum* L. (Sorsa *et al.*, 1988), coontail *Ceratophyllum demersum* L. (Smit *et al.*, 1990) and particularly for hydrilla *Hydrilla verticillata* (L.f.) Royle (Netherland and Shearer, 1996; Nelson *et al.*, 1998; Shearer and Nelson, 2002).

Applying a sublethal concentration of either fluridone or endothall in combination with a hydrilla-specific fungal pathogen, *Mycoleptodiscus terrestris* (Gerd.) Ostazeski, reduced hydrilla biomass by >90% and was considerably more effective than using the herbicide or pathogen alone (Netherland and Shearer, 1996; Nelson *et al.*, 1998; Shearer and Nelson, 2002). It was hypoth-

esized that the sublethal rate of either herbicide stressed the hydrilla plants by inhibiting growth and weakened their natural plant defence system, thus increasing their susceptibility to pathogen attack (Netherland and Shearer, 1996; Nelson *et al.*, 1998). For fluridone, the combination also reduced exposure time. Using either endothall or fluridone alone required higher rates that also caused injury to desirable native aquatic plants, including vallisneria (*Vallisneria americana* Michx.) and native pondweeds (*Potamogeton* spp.) (Nelson *et al.*, 1998; Shearer and Nelson, 2002). By using a lower rate of the herbicide with the pathogen, excellent selectivity was achieved and the damage to these non-target species was minimal.

## Prescribed burning and biological control agents

In many areas, prescription burning can be used to reduce the incidence of catastrophic wildfires (Briese, 1996) or to control invasive plants (DiTomaso *et al.*, 2006a,b,c). When used in combination with biological control, the timing of prescribed burning needs to take into consideration the reproductive capacity and life history of the BCAs. As with the combination of herbicides and biological control, some unburned patches should be preserved to maintain a refuge population of the BCAs (Briese, 1996).

For the integrated control of leafy spurge using burning, Fellows and Newton (1999) showed that burning conducted between mid-May and October did not have a detrimental effect on larval survival in *Aphthona nigriscutis* Foudras and increased the insect's establishment by more than twofold compared to unburned areas. During this timing interval, the adults were not active and the juveniles were below ground. The enhanced establishment of *A. nigriscutis* was due to an increase in colonization in the bare ground created by the burn. It was postulated that the litter layer interferes with reproduction of the BCAs. *A. nigriscutis* is considered difficult to establish in dense, mixed stands of leafy spurge and grass. Burning, however, increased the initial density of leafy spurge in the first growing season after the burn, which increased colonization through recruitment. However, the increase in the insect populations gave about seven times better control of leafy spurge than in the unburned site by the end of the first year (Fellows and Newton, 1999). Based on these results, the authors concluded that periodic burning of leafy spurge patches at the proper time of year would lead to expansion of the established colonies and provide earlier control of leafy spurge (Fellows and Newton, 1999).

A similar situation was also reported for common St. Johnswort (*Hypericum perforatum* L.). Prescription burning stimulated an increase in the crown density of the species through vegetative regrowth from rootstocks and lateral roots (Briese, 1996). Concomitant with this, St. Johnswort beetle (*Chrysolina quadrigemina* Suffrian) populations dramatically declined as a result of the fire. Interestingly, insect numbers quickly rebounded, primarily through influx of the beetle from adjacent non-burned sites. This increase in the beetle population in the burned site was associated with increased recruitment and fecundity due to higher available nitrogen and perhaps other nutrients that stimulated plant growth in the burn site. High foliar nitrogen levels in these St. Johnswort plants were associated with a population buildup of the beetle, which may have recognized the burn sites as favourable feeding areas (Briese, 1996).

Prescribed burning has been shown to be an effective tool for the management of yellow starthistle in California (DiTomaso *et al.*, 2006b). At the same time, several biological control insects are widespread throughout the state and there is some concern that prescribed burning may negatively impact the presence or activity of these organisms. A study conducted by DiTomaso *et al.* (2006b) showed no significant reduction in the attack rates of false peacock fly (*C. succinea*) in a burn site 1 year later. For hairy weevil (*E. villosus*), attack rates were high in both burned and adjacent unburned areas but were highest in the burned areas. Thus, despite the likely death of weevil larvae within the seed heads of yellow starthistle in the burned site the previous year, new recruitment of BCAs the following year was rapid.

## Plant competition and biological control agents

The competitive ability of a plant can be significantly compromised by the activity of biological control organisms. Insects that bore into roots, shoots and stems, defoliate the plant, destroy seeds or extract plant fluids can reduce the competitive ability of that plant with regard to its neighbouring vegetation (DiTomaso *et al.*, 2006c). Similarly, pathogens that infect the vegetation or underground parts can reduce the photosynthetic ability, water-mining capacity or vegetative growth of a species.

The density and cover of spotted knapweed in the western United States is generally lower in areas with higher grass competition (Müller-Schärer, 1991). Story *et al.* (2000) released the root-mining moth *A. zoegana* into two adjacent areas, one with high grass cover (~50%) and the other with low grass cover (~10%). After monitoring the buildup of moth populations, as well as the effect on the number of bolting spotted knapweed plants, they found that by the third year, the percentage of knapweed plants infested with *A. zoegana* in the high grass-cover plots was nearly twice that of the low grass-cover plots. This corresponded to a 50%

reduction in the number of bolted plants per unit area in the high-grass site compared to the low-grass site.

## Conclusion

Although there are few studies focused on combining biological control efforts with other weed management techniques, there are great opportunities to increase the efficiency of weed management strategies using integrated combinations. The potential exists for BCAs that may currently be considered ineffective when used alone to be successful when combined with other control techniques. The complexity of these systems, however, requires a comprehensive understanding of the biology and ecology of the organisms, appropriate timing of the treatments and the long-term effects. Over time, integrated management approaches may provide more effective and economical options compared to the use of single management techniques.

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# Biological control of *Melaleuca quinquenervia*: goal-based assessment of success

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*Summary*

Success means different things to different people. Unfortunately, the success or failure of weed biological control projects is often evaluated by nonparticipants lacking knowledge of the original goals set by project architects. Criteria for success should match objectives and goals clearly articulated so that success can be properly archived for future synthesis. The Australian tree *Melaleuca quinquenervia* (Cav.) S.T. Blake, an aggressive invader of the Florida Everglades, may be the largest plant ever targeted for biological control. We realized early on that biological control agents would not remove the many tons of woody biomass that comprised these infestations and so would be unlikely to reduce the infested acreage. Control of this plant by other means, however, was complicated by the billions of canopy-held seeds that are released following injury to the tree. A plan was developed in coordination with land management agencies wherein the goal of biological control was to curtail melaleuca expansion and suppress regeneration while using other means to remove mature trees. Three insect species have been released and others are under consideration. These agents, supplemented by the impacts of an adventive rust fungus and a scale insect, have met established goals and this project shows signs of an emerging success based on the established goals.

***Keywords***: Everglades, invasive plants, habitat restoration.

## Introduction

'Success has many fathers while failure dies an orphan'. This oft-quoted aphorism illustrates the political necessity of highlighting successes when they occur so that one's endeavors continue to be supported in the future. Unfortunately, weed biological control projects are rarely undertaken based on the likelihood of a successful outcome (Peschken and McClay, 1995). Instead, biological control is often the method of last resort after other methods against recalcitrant weeds have failed. This is not conducive to improving the overall statistical success rate but is often the most responsible or economic option. Biological control of many serious weed problems would likely never be attempted if target choice was based primarily on maximizing the probability of success as advocated by Peschken and McClay (1995).

Many investigators have focused on the performance of individual agents to gauge success, primarily with an aim toward predicting which taxonomic groups make the best biological control agents. Such post-hoc analyses suggest a low success rate for weed biological control, with only a small proportion of successfully established agents producing effective control (Crawley, 1989a,b). Critics have used these statistics to advise against the use of biological control as a weed management tool (Louda and Stiling, 2004). McFadyen (1998, 2000), however, strongly disagreed with this advice and emphasized the need for project-based assessments.

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Most authors use the term 'success' to refer only to 'complete success', wherein no other measures are needed to reduce the weed populations to acceptable levels. However, this neglects the importance of partially successful projects that have value when less effort is subsequently required to control the weed, because the density or extent of weed populations is reduced, or the weed is less able to reinvade cleared areas or is slower to disperse (Hoffmann, 1995). Success and failure are at the extreme ends of a continuum of many possible outcomes and even moderate amounts of stress can reduce the competitive ability of a weed and render it less invasive (Center *et al.*, 2005; Coetzee *et al.*, 2005).

Successful biological control agents often act by preventing continued expansion of a weed population, rather than by reducing population densities (Hoffmann, these proceedings). Hoffmann also noted that it may be necessary to model weed outbreaks that never happen to perceive biological control effects. Documentation of such effects is difficult, at best, which explains why so many projects are incompletely evaluated and even successful projects may be undervalued or forgotten. Thus, statistical success rates should be viewed with circumspection, inasmuch as only obvious successes are reported. Furthermore, weed declines may occur incrementally over many years or even decades and may not be easily observed, especially when observational baselines shift over time, project funding terminates, or personnel changes interrupt collection of critical data.

Success of projects should be assessed in terms of the project's original goals and objectives. Hence, a project can and should be deemed successful whether or not the density of the weed is reduced so long as the goals set out by the project architects are met. In this sense, it is possible to have complete success without complete control so long as the project goals are clearly stated, understood and documented. A recent project aimed at the control of *Melaleuca quinquenervia* (Cav.) S.T. Blake (melaleuca) in South Florida as part of a broader Everglades restoration effort is used herein to illustrate this concept.

## The target

Melaleuca is a large tree (up to 30 m tall) of Australian origin that was introduced into southern Florida during the latter part of the 19th century (Dray *et al.*, 2006). It has invaded wetland habitats, especially fire-maintained Everglades ecosystems, where the burning regime now favours melaleuca over less fire-tolerant native species. As a result, vast areas of these heterogeneous marshes have been transformed into swamp forests consisting of melaleuca monocultures. Melaleuca rapidly dominates infested areas after its initial colonization (Laroche and Ferriter, 1992) and at its peak was estimated to occupy at least 607,000 ha of conservation lands in the southern part of Florida (Bodle *et al.*, 1994).

Control of melaleuca is complicated by the fact that it grows in areas that are hazardous and strenuous to work in and where access is difficult. These difficulties are exacerbated by the tree's reproductive biology. Melaleuca flowers numerous times each year, often several times on the same stem axis due to indeterminant growth, forming spike-like clusters composed of multiple, dichasial groups of three flowers each (Tomlinson, 1980). Each cluster contains up to 75 individual flowers. Fruits arising from these flowers are persistent serotinous capsules that each contains 200–350 minute seeds (Meskimen, 1962). These generally remain in the fruits until disruption of the vascular connection causes the capsules to desiccate and open, often *en masse*, after a fire, freeze, drought, or herbicide treatment (Meskimen, 1962). A few (about 12% per year) open continuously, as radial growth of the stem separates the vascular connection, producing a light, perpetual seed rain of about 3 billion seeds/ha/year (M. Rayamajhi *et al.*, unpublished data). Seeds that fall to the ground form a rather short-lived soil seed bank with a half-life of less than 1 year (Van *et al.*, 2005). A single large tree located within a dense stand retains about 50 million seeds in its canopy with stands holding as many as 25 billion seeds/ha (M. Rayamajhi, unpublished data). An isolated tree may hold twice as many seeds as one of similar size in a dense stand. Surprisingly, a large proportion (85–90%) of these are actually hollow seed coats (Rayachhetry *et al.*, 1998; Rayamajhi *et al.*, 2002). Nonetheless, the remaining 10–15% of embryonic seeds create an enormous regenerative capacity capable of producing seedling densities of up to 2256 seedlings/m$^2$ (Franks *et al.*, 2006) following a massive simultaneous seed shed induced by fire, drought, or herbicide application. These may grow into thickets of up to 130,000 small trees/ha (Van *et al.*, 2000). As the stand matures, it thins to about 8000–15,000 trees/ha comprised mostly of mature trees with an understory of suppressed saplings (Rayachhetry *et al.*, 2001). The standing biomass in these forests has been estimated at 129–263 metric tonnes (t)/ha (Van *et al.*, 2000).

Isolated individual trees constitute a seed source for further encroachment. The seeds, when released, generally fall within 15 m of the parent tree (Meskimen, 1962). They often grow into dome-shaped clumps or 'heads' with the parent trees in the centre and progressively younger trees toward the periphery. These eventually coalesce with others blanketing vast acreages of wetlands with dense swamp forests. The isolated 'outliers' therefore are regarded as potential new infestations and, as part of a quarantine strategy, are first priority for control operations (Woodall, 1981).

The trees within these stands produce multiple adventitious roots that form an intertwined skirt at the waterline or on saturated soil (Meskimen, 1962). These contribute biomass to the forest floor and trap large amounts of litterfall as well as organic debris causing soil accretion (White, 1994), thus increasing the local

elevation (T. Center, personal observation). Altering the elevation of the Everglades even by a few centimeters dramatically shifts plant community composition (Ogden, 2005), thus these newly created melaleuca islands forever change the physiography and ecology of the area. There is also evidence that essential oils in melaleuca litter may be allelopathic (Di Stefano and Fisher, 1983). These changes render infested habitats unsuitable for many native species making restoration difficult if not impossible.

## The melaleuca management plan

The South Florida Water Management District in conjunction with the Exotic Pest Plant Council convened a meeting of the major agencies that were managing the melaleuca problem. They developed a 'Melaleuca Management Plan for Florida', published during 1990 and revised in 1994 and 1999.

Two points were evident during the development of this plan. First, biological control could not eliminate the huge amounts of woody biomass present; herbicidal and mechanical control would therefore be needed to reduce the infestations to a maintenance level. Second, public agencies could not expend public funds to control melaleuca infestations on private lands that often abutted cleared tracts of public lands. These unassailable stands provided an invasion front and a potential seed reservoir to support reinvasion of cleared sites. The role of biological control in this plan was to neutralize the reproductive potential of these remaining stands by reducing seed production, seedling recruitment and regeneration; thereby inhibiting spread, reducing reinvasion of cleared areas and facilitating traditional control measures. However, implementation of biological control would take time, whereas chemical and mechanical control could be employed rapidly. So the plan relied on an early deployment of traditional control measures that would gradually be supplanted by biological control as agents became available (Figure 1).

## The biological control agents

Insects associated with melaleuca were enumerated in Australia during the late 1980s and early 1990s (Balciunas, 1990). These inventories revealed an entomofauna of over 400 species (Balciunas, 1990; Balciunas *et al.*, 1993a,b, 1994, 1995a,b,c; Burrows *et al.*, 1994, 1996). The most promising species were studied further and three have now been released.

The first insect evaluated was the weevil *Oxyops vitiosa* Pascoe (Purcell and Balciunas, 1994). This insect, being a flush feeder on growing stem tips, was desirable because of its ability to disrupt flower production, which depends on continual growth of the stem axis. It proved to be host-specific (Balciunas *et al.*, 1994; G. Buckingham, unpublished report) and was released during 1997 (Center *et al.*, 2000). Its need to pupate in dry soil (Purcell and Balciunas, 1994; Center *et al.*, 2000), however, limited it to habitats that were not permanently under water. Field and laboratory assessments of a mirid, *Eucerocoris suspectus* Distant, in Australia (Burrows and Balciunas, 1999) suggested that its host range was limited to melaleuca and a few close relatives. Follow-up studies in US quarantine facilities failed to confirm this so it was dropped from consideration. The host range of the pergid sawfly *Lophyrotoma zonalis* (Rohwer) proved sufficiently narrow (Burrows and Balciunas, 1997; Buckingham, 2001), but after discovering that larvae synthesize toxic octapeptides

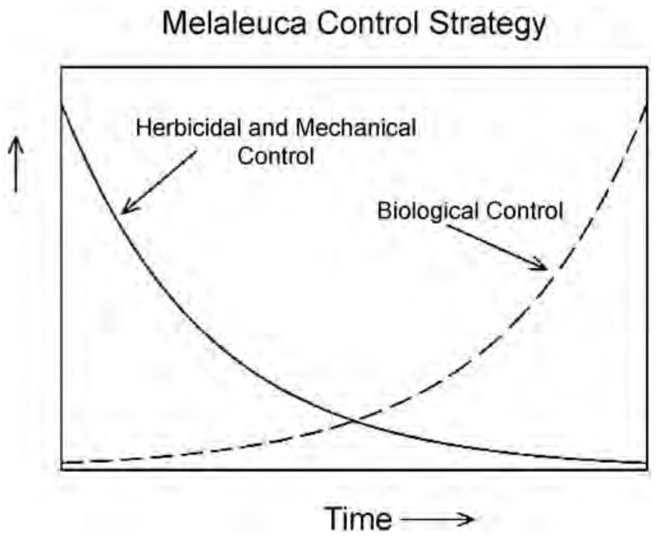


**Figure 1.** The strategy employed to control melaleuca in south Florida involved early use of traditional control methods to reduce biomass while biological controls were being developed and implemented.

(Oelrichs *et al.*, 1999), we elected not to release it out of concern over potential negative effects to insectivorous wildlife.

The melaleuca psyllid *Boreioglycaspis melaleucae* Moore was found to be host-specific (Purcell *et al.*, 1997; Wineriter *et al.*, 2003), and was released during 2002 (Center *et al.*, 2006, 2007). It feeds mainly on the new growth but will also utilize older leaves and the green stems. Furthermore, it completes its life cycle entirely on the plant so it is less restricted by habitat. The tube-dwelling pyralid *Poliopaschia lithochlora* (Lower) was highly rated because of its ability to damage melaleuca and its preference for low-lying, humid habitats (Galway and Purcell, 2005), but its use of an ornamental species, *Melaleuca viminalis* (Sol. ex Gaertner) Byrnes, during testing diminished its prospects (M. Purcell, unpublished data). A fergusoninid gall fly, *Fergusonina turneri* Taylor, and its mutualistic nematode *Fergusobia melaleucae* Davies and Giblin-Davis, also proved to be highly specific (Giblin-Davis *et al.*, 2001) and were first released during 2005 (Blackwood *et al.*, 2006). It has proven difficult to establish but efforts are continuing. Most recently a stem-galling cecidomyiid, *Lophodiplosis trifida* Gagné, has proven to be host-specific (S. Wineriter *et al.*, unpublished data) and should gain approval for release. A bud-feeding weevil *Haplonyx multicolor* Lea and a leaf-galling cecidomyiid *Lophodiplosis indentata* Gagné are currently under consideration.

Two adventive organisms have also recently infested melaleuca trees in Florida. A pestiferous, undescribed scale insect (Pemberton, personal communication) was detected in Florida during 1999. It attacks melaleuca trees as well as some 300 other plant species (Pemberton, 2003; R. Pemberton, unpublished data). The guava rust *Puccinia psidii* G. Winter (Basidiomycetes: Uredinales), which infects mainly young foliage, appeared during 1997 (Rayachhetry *et al.*, 1997) and is now widespread.

## The effects of the biological control agents

Numerous studies aimed at determining the impacts of *O. vitiosa* and *B. melaleucae* have been conducted or are ongoing. However, determinations of the individual effects of one have been confounded by the presence of the other, as well as by the presence of the adventive rust fungus and scale insect. These studies have included comparisons of melaleuca stands with and without the agents, caging studies, defoliation experiments, insecticide and fungicide exclusion experiments, and before and after comparisons of stand dynamics.

### Flower and seed production

The effects of herbivory by *O. vitiosa* on melaleuca performance were possible early during the release program when none of the other organisms were present. Pratt *et al.* (2005) compared flowering frequency in melaleuca stands where the weevil had been released to stands without them. They found that the likelihood of flowering increased with tree size but that undamaged trees were 36 times more likely to reproduce than damaged trees in similar habitats (Figure 2). Overall, about 45% of the weevil-free trees were flowering compared to about 2% of infested trees.***

In another study, Pratt *et al.* (2005) enclosed the canopies of small (2.9 cm diameter at breast height or

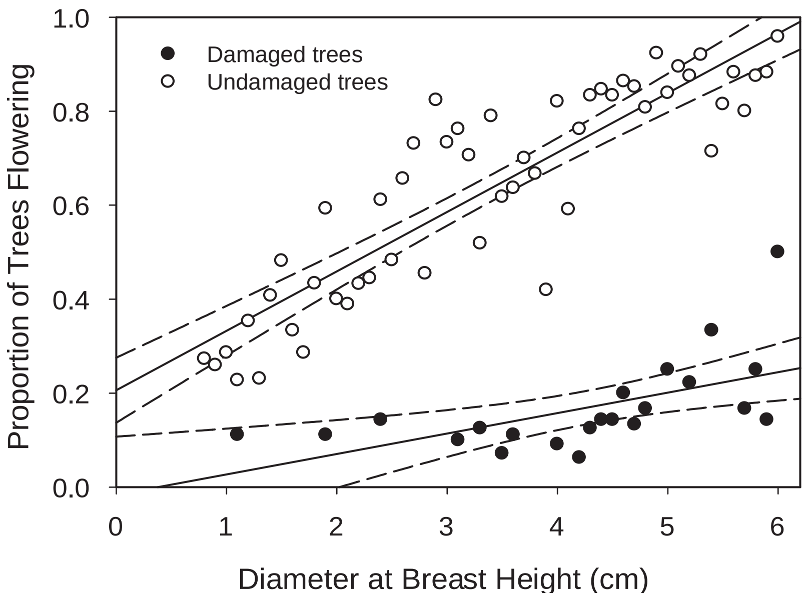


**Figure 2.** Release of the weevil *Oxyops vitiosa* profoundly affected flowering of melaleuca trees. The proportion of the trees that produced flowers was much lower after being damaged by the weevils regardless of size.

dbh) trees with sleeve cages and introduced weevil larvae into the enclosures, either once or twice, to produce one or two defoliations of the young foliage. The second defoliation was done about 10 weeks after the first. These treatments were compared to controls with no defoliation or to trees artificially defoliated by manually removing all foliage. Flower production on all trees was monitored monthly for 1 year. The control trees flowered normally during this period, whereas trees artificially defoliated failed to produce any flowers. Trees defoliated once or twice by the weevil larvae produced a few flowers but numbers were not statistically different from each other or from the artificial defoliation treatment (Figure 3).

Interestingly, in a comparison of ten herbivore-impacted trees with ten non-impacted trees at similar, nearby sites at Estero, Florida, Rayamajhi *et al.* (unpublished data) found that herbivory by *O. vitiosa* resulted in higher rates of capsule abortion when compared to sites without natural enemies. Mean number of capsules in herbivore-impacted infructescences was reduced by nearly 50% compared to the herbivore-absent site. This decreased density of capsules was apparent as gaps in the capsule clusters caused by abortion of the undeveloped fruits. The herbivore-impacted trees were very similar to those near Brisbane, Australia where the average infructescence was 5.7 cm long but contained only 18 capsules (Rayamajhi *et al.*, 2002). The average numbers of seeds per capsule were similar in both the Florida and Australian sites.

Rayamajhi *et al.* (unpublished data) have also found that when the trees were subjected to attack by *O. vitiosa*, the percentage of embryonic seeds decreased, as did seed viability and germination ability. Seed viability and germination tests (Van *et al.*, 2005) also revealed reductions in both measures of seeds from herbivore-attacked trees compared to controls.

## Seedling survival

Franks *et al.* (2006) described the effects of the weevil larvae and the psyllids, alone and in combination, on growth and survival of melaleuca seedlings by caging the insects on 26 cm-tall seedlings in field plots. They compared these results to a natural infestation of the insects on nearby seedlings. *O. vitiosa* larvae had no effect on seedling height, leaf number, or survival, whereas psyllids caused all of these measures to decrease by about 55–60% over the 5-month term of the study. About 95% of seedlings survived when protected from psyllids as compared to only 40% when exposed to herbivory (Center *et al.*, 2007).

In another study, Tipping *et al.* (unpublished data) found that after becoming infested by both the psyllid and the weevil, melaleuca trees recruited a much lower density of seedlings than trees without either herbivore. They also compared densities of saplings in plots 9 $m^2$ that were periodically treated with insecticide to exclude herbivory to saplings in untreated plots. The plots were located in an area that had burned during June 1998, resulting in a massive seed rain and thickets of about 1000 seedlings/$m^2$. By the time the study was initiated during 2002, these had become saplings and had grown to about 70 cm in height. Densities in

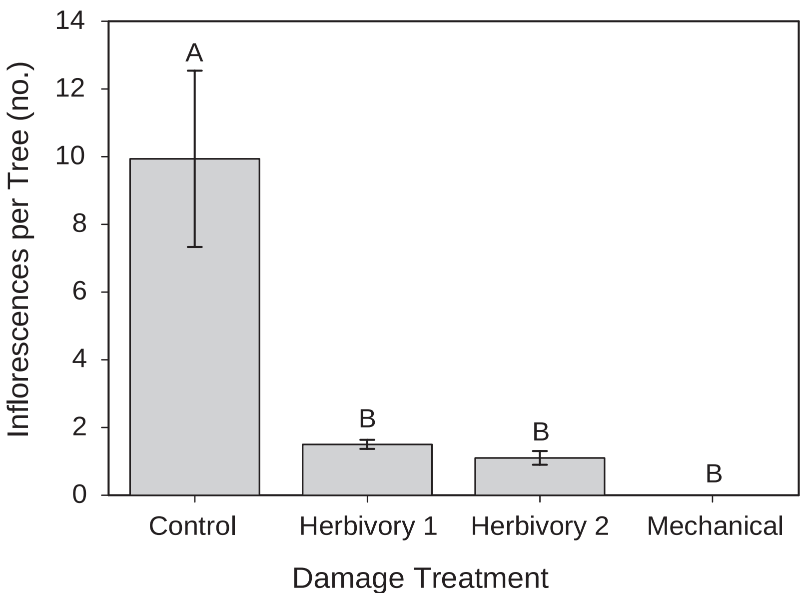


**Figure 3.** Small trees were caged then subjected to herbivory by *Oxyops vitiosa* either once (Herbivory 1) or twice (Herbivory 2) or to mechanical defoliation and compared to undefoliated controls. Defoliated trees, regardless of the manner of defoliation, produced very few flowers relative to the controls.

the protected plots were virtually unchanged during the 5-year period of the study as compared to those in the unprotected plots which declined by almost half.

## Sapling growth

Tipping *et al.* (unpublished data) conducted two insecticide exclusion studies on the growth of melaleuca saplings in common garden experiments over about a 3-year period. The first experiment investigated the effect of the melaleuca weevil, *O. vitiosa*, and supplemental irrigation on the growth of small trees. The second examined the effects of herbivorous insects (both the psyllids and the weevils) and plant chemotype (nerolidol or viridifloral). In both cases, plants treated with insecticide more than doubled in stature, whereas those not treated grew very little. In the first study, plants attacked by *O. vitiosa* grew at a much slower rate compared to the protected plants (Figure 4). The unprotected plants produced more stem tips per unit of height, creating a shorter, bushier habit, which provided more resource for the tip-feeding insects. Supplemental irrigation improved the growth of insecticide-treated trees but had no effect on trees that were not treated with insecticide. Chemotype had no apparent effect on the impact of the insects. Seed capsule production was much lower among unprotected plants in both studies.

## Stand dynamics

Rayamajhi *et al.* (2007) studied the dynamics of melaleuca stands before and after the widespread impacts of the biological control agents. They found that the average density of the trees in mature stands declined by 72% overall from 15,800 trees/ha during 1996 to 4400 trees/ha during 2003. Interestingly, the standing biomass based on harvesting studies increased somewhat from an initial average of 263 t/ha to 274 t/ha during the latter harvest. This was because most of the mortality was among the smaller suppressed trees in the understory that represented a small proportion of the biomass. The density of small trees, those with a dbh of less than 10 cm, decreased 83% from 12,600 to 2200 trees/ha; density of intermediate-sized trees with a dbh of 10–20 cm decreased 46% from 2600 to 1400 trees/ha; density of large trees >20 cm increased from 600 to 800 trees/ha.

Another study (Rayamajhi *et al.*, 2007) showed that densities decreased between 1997 and 2006, in part due to self-thinning. The decline accelerated after the effects of biological control became apparent and the rate of decline was inversely related to the position of the trees within the stands. Densities of trees at the periphery, which consisted mostly of small individuals, decreased by about 6076 individuals/ha/year before 2001

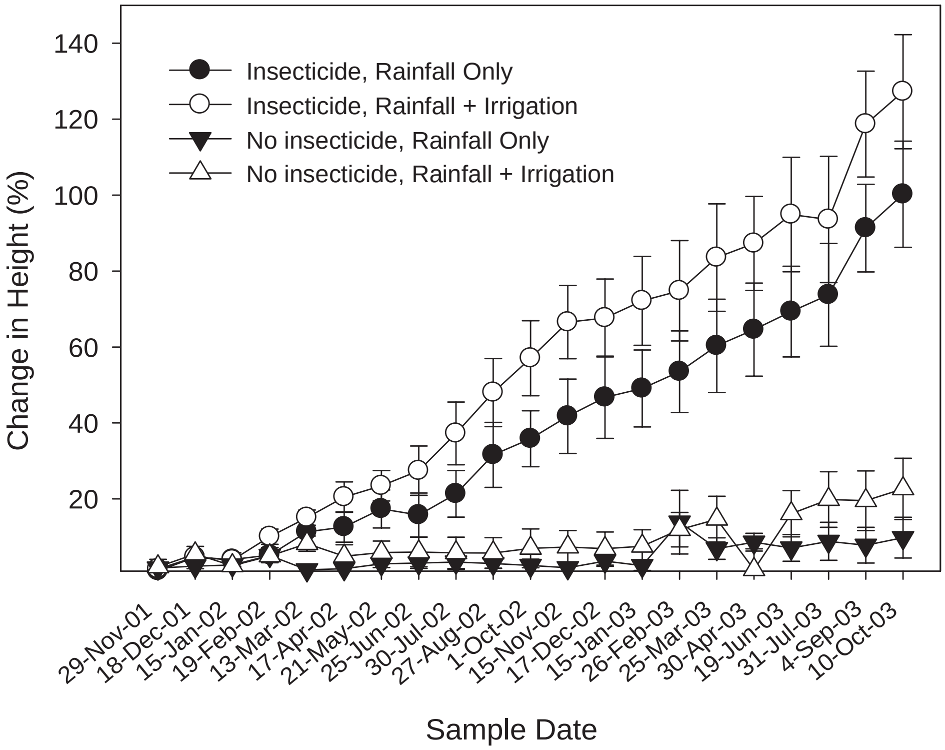


**Figure 4.** Small trees grown in a common garden plot were treated with insecticide to exclude herbivorous insects and given supplemental irrigation and compared to unprotected and unwatered trees. Protected trees grew vigorously and those receiving supplemental irrigation grew the most. Unprotected trees grew very little and the supplemental irrigation seemed to have little effect.

as compared to 16,725 individuals/ha/year after 2001. Densities in the inner portions of the stands, which contained higher proportions of larger trees, decreased at relatively constant rates. This further demonstrated the greater effect of herbivory on smaller trees. The average diameter of the trees increased, not because they grew but because of selective mortality of smaller individuals. This was corroborated by a decrease in or leveling off of total basal area coverage during the post-release period in contrast to a prior increasing trend.

Despite the finding that the surviving larger dominant trees accounted for most of the biomass, biomass allocation changed due to extensive defoliation of all of the trees. The foliage of large trees growing in dense stands was limited to the upper branches at the treetops and this accounted for only 5.1% of the total biomass during 1996, before insect-induced defoliation. This decreased from 17 to 8 t/ha to represent only 1.5% of the total biomass during 2003 (Figure 5). The biomass allocated to seed capsules decreased by 85% from 6.7 t/ha, or 0.46% of the total biomass to 1.0 t/ha, or 0.29% of the total biomass.

Litter-traps were placed under mature melaleuca stands to collect leaf litter in an attempt to measure the activity of the biological control agents in the canopy of taller trees. The proportion of fallen leaves that exhibited weevil damage symptoms was analysed. Though the weevil releases began in 1997, the first weevil-damaged leaves did not appear in the traps until 1999 (represented by 5% of the trapped leaves) and by 2005, the proportion of damaged leaves reached approximately 45% (Rayamajhi *et al.*, 2007). This increased percentage of damaged leaves reflected the decreasing proportions of leaf biomass (stem to leaf biomass), increasing tree mortality, and decreasing tree densities.

## Stump regrowth

The ability of melaleuca to sprout from cut stumps complicates control. This requires follow-up herbicide treatment to prevent coppicing and stand regeneration. Several studies have indicated that the flush of foliage associated with this regrowth is highly attractive to both psyllids and weevils, as well as to the rust fungus. Pratt *et al.* (unpublished data) found that insecticide exclusion of biological control agents led to an increase in leaf and stem biomass compared to unprotected stumps (Figure 6). Chronic attack over an 18-month period led to mortality of almost half the unprotected plants. In a similar insecticide- and fungicide-exclusion study, Rayamajhi *et al.* (unpublished data) found that the rust fungus, *P. psidii*, played an important additive role. Proportion of photosynthetic tissues and the mortality of stems were higher in treatments involving both insects (*O. vitiosa* and *B. melaleucae*) and rust (*P. psidii*) together than in treatments using either alone. Death of the regrowth often led to the death of the stump itself (M. Rayamajhi *et al.*, unpublished data). These data indicate that biological control can compliment, and in some cases replace, the use of herbicides for stump treatment.

# Discussion

Clearly, many melaleuca stands have undergone significant declines and remaining trees are now in poor condition. However, vast stands of melaleuca still exist that overtly appear unchanged. Yet after closer scrutiny, we have revealed that the dynamics of these stands have changed in very significant ways. Fewer trees now produce flowers, those that do flower produce

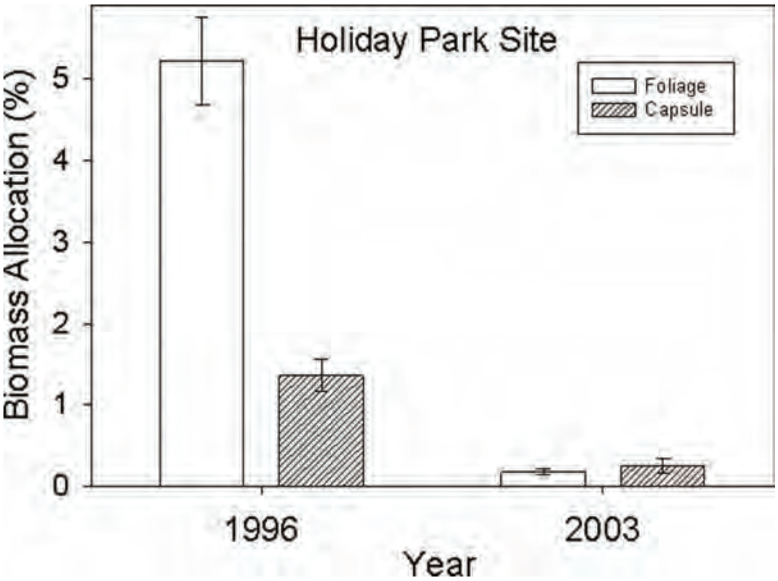


**Figure 5.** The proportion of the total tree biomass allocated to foliage declined dramatically due to defoliation primarily by *Oxyops vitiosa* and the psyllid *Boreioglycaspis melaleucae*.

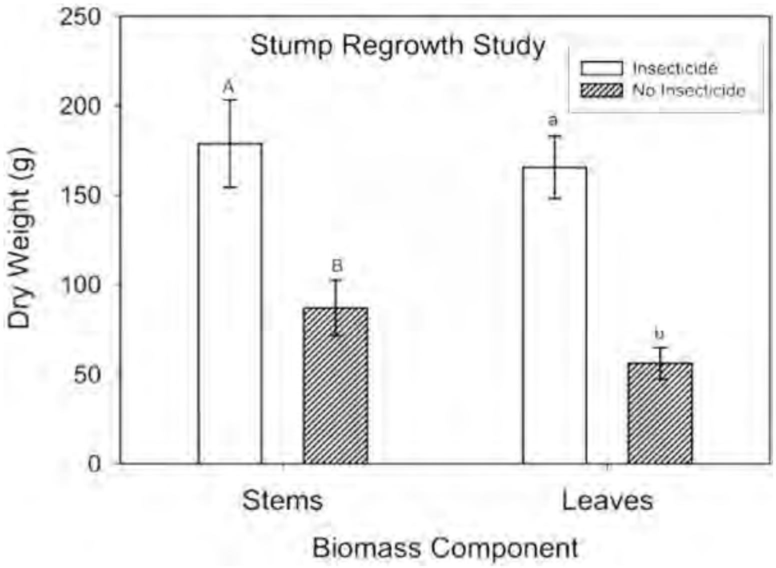


**Figure 6.** Regrowth from stumps, reflected by the biomass of stems and leaves produced after felling of the original trees, was substantially more when regrowth was treated with insecticide thus reducing the effects of the weevils and the psyllids.

fewer inflorescences and the inflorescences produced contain fewer individual blossoms. Many of the fruits abort and those that do manage to set seed produce a smaller proportion of viable seeds. The constant defoliation of the stem tips causes the capsules to desiccate and release seeds during drier periods when conditions are unfavourable for germination. Those that do fall, lodge in a favourable site and manage to germinate are infested by psyllids that kill a large proportion before they attain a significant size. If they survive, they grow slowly due to constant defoliation and produce few flowers. Meanwhile, existing stands have nearly been removed from publicly held lands and those on private lands are less invasive. Hence, the goals of the project, as stated above, have been met so the project should be considered a success. It is not yet a 'complete' success in that biological control is more effective in some habitats and during some periods than others but additional agents that are currently under development may fill these gaps.

## Acknowledgements

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# *Hydrilla verticillata* threatens South African waters

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*Summary*

South Africa's inland water systems are currently under threat from hydrilla, *Hydrilla verticillata* L. Royle (Hydrocharitaceae), the worst submerged aquatic weed in the USA. The presence of the weed was confirmed for the first time in South Africa in February 2006, on Pongolapoort Dam in KwaZulu-Natal. An aerial survey revealed that the infestation on this dam covers approximately 600 ha, which is far greater than initially thought. Despite reports that it may be present in other water bodies, surveys have shown that it is restricted to Pongolapoort Dam. We conducted a boater survey which showed that there is significant potential for this devastating weed to spread beyond Pongolapoort Dam, and containment of hydrilla is of utmost priority. Research into the suitability of the already established biological control agents, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock (Diptera: Ephydridae), from the USA, as potential agents in South Africa, is also being conducted. However, the South African hydrilla biotype is different from the biotypes in the USA, and this needs to be borne in mind when considering which agents to release.

***Keywords***: potential spread, management, genetic analysis.

## Introduction

The confirmation of *Hydrilla verticillata* L. Royle (Hydrocharitaceae) (hydrilla) in South Africa from Pongolapoort Dam, KwaZulu-Natal province (KZN), in early 2006 (L. Henderson, personal communication, 2006) prompted immediate action to contain and control this weed, and prevent further spread to other water bodies around South Africa. At present, it appears that hydrilla is restricted to Pongolapoort Dam, which is the centre of a multimillion rand tourist industry. It is imperative that we gain an understanding of the dynamics of the hydrilla invasion in South Africa, and potential for its control, because there is a knowledge gap in South Africa surrounding submerged aquatic plants, particularly from a biological control aspect.

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## Current distribution of hydrilla in South Africa and potential for spread

Hydrilla is one of the most problematic submerged plants worldwide, invading both tropical and temperate regions because of its tolerance to a wide range of environmental conditions (Cook and Lüönd, 1982). It is not clear how or when hydrilla entered South Africa, and so the first step in the hydrilla biocontrol programme in South Africa was to determine the extent of its distribution. Following reports that hydrilla's presence was suspected in a number of water bodies in KZN, both aerial and boat surveys were undertaken, which confirmed that hydrilla is currently restricted to an area of about 600 ha in Pongolapoort Dam. However, heavy rains in early January 2007 resulted in the flooding of this area, and the dam increased in capacity from 73% to 92% full in 1 week. The possibility that hydrilla has spread throughout the dam, and into the Pongola River below should not be ruled out, and warrants further investigation.

While containment of hydrilla in Pongolapoort Dam is currently the main control strategy in South Africa, there is potential for this plant to spread. In the USA, the main mode of spread of the weed is via recreational

boaters and fishermen (Balciunas *et al.*, 2002) as fragments of the plant get caught in anchors and propellers and are then transported between water bodies. It is therefore very likely that hydrilla may spread throughout South Africa in this manner, particularly because Pongolapoort Dam attracts thousands of tourists annually, and because it is home to the annual Tiger Fishing Festival, the biggest tiger fishing competition in the southern hemisphere that attracts fishermen from all over South Africa, and neighbouring Swaziland and Mozambique.

Fishermen are primarily responsible for hydrilla's spread in the USA. Therefore we conducted a survey at the annual Tiger Fishing Festival in September 2006 to determine the potential for hydrilla to spread throughout South Africa by assessing boating behaviour of the fishermen, and whether they were aware of the presence of hydrilla on the dam. One hundred sixty-three fishermen were asked questions from a structured questionnaire. The results showed that 51% of the fishermen interviewed only used their boats once on Pongolapoort Dam, and that was at the September competition. The results also showed that 14.1% of the fishermen used their boats only on Pongolapoort Dam between January 2005 and September 2006, and 20 respondents (12.2%) never used their boats anywhere except once at the 2006 fishing competition, between January 2005 and September 2006.

However, analysis of the number of times fishermen used their boats in South Africa highlighted that dams outside of KZN were visited more frequently than those in KZN, and the majority of fishermen traveled between 200 and 800 km to reach their fishing destinations, emphasizing the potential for hydrilla to spread around South Africa. Even though a containment strategy is in place on Pongolapoort Dam, this survey stressed that more water bodies in South Africa need to be assessed for the presence of hydrilla as a result of boating activities before the fishing competition in September.

## Management options

Mechanical and chemical control has been the most widely used control methods in the USA, although their success is varied. Typically, mechanical control is time-consuming and only offers temporary control, and its use has been dissuaded in South Africa, particularly because new infestations can result from plant fragments. Until a biocontrol programme can be implemented, chemical control is currently the most favourable option for hydrilla. It should be controlled using herbicides as soon as possible because it is confined to only one system. The most effective herbicide to date against hydrilla in the USA is fluridone, which has been widely used for large-scale control (Dayan and Netherland, 2005). Trials with this herbicide will commence as soon as it is imported into South Africa.

Fluridone does have non-target side effects on other aquatic vegetation and fish, which has led to sublethal doses being applied as this minimizes these effects, and is more cost-effective. However, hydrilla has become resistant to these doses in the USA, which has complicated control programmes (Michel *et al.*, 2004; Dayan and Netherland, 2005). So it becomes a risk–benefit issue in South Africa – is it worth using a lethal dose that will remove large amounts of the plant, but that will impact the fauna and flora in the dam, against the potential of resistance developing if sublethal doses are used, thereby ruling out the most effective control strategy against it?

The most sustainable long-term strategy to control hydrilla should be biological control. The option of using the two species of ephydrid flies, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock (both Diptera: Ephydridae) as potential control agents against hydrilla in South Africa is being investigated, because these are the only two agents that have established in the USA (Center *et al.*, 1997; Bennett and Buckingham, 2000; Grodowitz *et al.*, 2000; Wheeler and Center, 2001). In addition, the weevil, *Bagous hydrillae* O'Brien (Coleoptera: Curculionidae), which was tested and released in the USA to control hydrilla but never established because it requires periods of drought for pupation (Grodowitz *et al.*, 2000), is being considered as a control agent in South Africa because drawdowns are implemented on Pongolapoort dam. Permits have been granted to import the flies and the weevil into South Africa from the USA, so that host specificity testing may commence.

Expanded surveys are also being conducted by both the USDA-ARS and CSIRO to find additional control agents for hydrilla in the USA (Overholt and Wheeler, 2006), following the discovery that infestations of hydrilla are resistant to fluridone (Michel *et al.*, 2004; Puri *et al.*, 2007). Surveys in Burundi, Uganda and other central and east African countries, and in Sumatra and China have found promising agents (Overholt and Wheeler, 2006), which could be considered as additional control agents in South Africa.

## Identification and origin of the introduced biotype using chloroplastic markers

Hydrilla is a widely distributed species whose range extends from New Zealand and Australia, through Southeast Asia, north through China, into Siberia, and west into Pakistan, and it also has a local and disjointed range in Africa and northern Europe (Cook and Lüönd, 1982). Studies have identified more than 28 different hydrilla biotypes, which could have important consequences for biological control of the plant in South Africa. Four major biotype clusters and one minor outlier cluster have been identified (Madeira *et al.*, 1997,

1999). The USA has two hydrilla biotypes – a dioecious strain that clusters closely with an Indian strain, and a monoecious strain that clusters closely with an accession from Korea (Madeira *et al.*, 1997).

Several biocontrol agents have been released in the USA, but only the leaf mining fly *H. pakistanae* is causing significant damage (Wheeler and Center, 2001). Regional variation in both the host plant and the control agent populations could potentially affect the effectiveness of new releases. It is therefore essential in any biocontrol programme to know which biotype is being dealt with to maximize the efficacy of control agents, by selecting agents from the same area as the plant biotype.

Therefore, samples of South African hydrilla were analysed using the *trnL* intron and *trnL-F* intergenic spacer of the chloroplast to determine to which major cluster of worldwide hydrilla the South African hydrilla belongs (Madeira *et al.*, 2007). In the sequencing it was identical to Malaysian and Indonesian samples. This biotype is also monoecious, and produces copious numbers of flowers, pollen, seedpods and seeds. South African hydrilla is therefore very different from hydrilla in the USA, and the control agents currently in use in the USA might not be as suitable to the biotype in South Africa.

Biotype analysis is also interesting from an introduction point of view. By determining to which cluster hydrilla belongs, inferences about how it was introduced to South Africa can be made. Hydrilla was introduced into the USA via the aquarium trade (Schmitz *et al.*, 1991), and it is likely that this was also the mode of introduction into South Africa. Interestingly, the majority of aquarium plants imported into South Africa come from Singapore, Malaysia, which is where the South African hydrilla biotype is most closely related.

## Conclusions

Much progress has been made in understanding the biology of hydrilla, the nature of the infestation and the potential for the weed to spread further in South Africa. We also know what the biotype is, but little is known about the flowering and reproductive phenology of this biotype in South Africa. This calls for further study in both the laboratory and the field in the upcoming months. Furthermore, the certainty that the *Hydrellia* flies and *Bagous hydrillae* will be suitable against the South African biotype cannot be guaranteed. It seems that the best way forward would be for South Africa to undertake surveys in Sumatra and China, and other Southeast Asian countries such as Thailand, Indonesia and Malaysia, from where the biotype originates.

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# Status of the biological control of banana poka, *Passiflora mollissima* (aka *P. tarminiana*) in Hawaii

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*Summary*

Surveys were conducted between 1982 and 1995 on banana poka, *Passiflora mollissima* Bailey (also known as *P. tarminiana*, subgenus *Tacsonia*) and related species in the Andes Mountains of South America. The objective was to identify potential biocontrol agents for control of banana poka in Hawaii, USA. Host-related insect diversity was greatest in Colombia, Ecuador, Peru and Venezuela, and poorest in Bolivia and Chile. Insect species observed represented eight orders, 35 families and approximately 67 species. Fifteen species were evaluated as potential biocontrol agents, of which five received in-depth testing. Two moths, *Cyanotrica necyria* Felder and Rogenhofer (Lepidoptera; Notodontidae) and *Pyrausta perelegans* Hampson (Lepidoptera; Pyralidae), were approved and released in Hawaii in 1988 and 1991, respectively; however, *C. necyria* did not establish and *Pyr. perelegans* established but has had negligible impact. A third moth, *Josia fluonia* Druce, *J. ligata* group (Lepidoptera; Notodontidae), had been approved for release and two flies, *Dasiops caustonae* Norrbom and McAlpine (Diptera; Lonchaeidae) and *Zapriothrica* nr. *salebrosa* Wheeler (Diptera; Drosophilidae), were undergoing final evaluation when the programme was terminated. A pathogen, *Septoria passiflorae* Syd., was released in 1996, with mixed results. Banana poka remains a serious weed pest in Hawaii.

***Keywords***: foreign exploration, South America, *Passiflora tripartita*, *Passiflora tarminiana*, weed biological control.

## Introduction

Banana poka, *Passiflora mollissima* Bailey (also referred to in literature as *P. tripartita* and *P. tarminiana*), was introduced into Hawaii from South America as an ornamental around the year of 1900, but escaped domestication to become a major forest weed in the upper elevation mountain rain forests (La Rosa, 1984). *P. mollissima* vines form dense mats of vegetation that cover understory plants and climb into overstory canopies of Koa, *Acacia koa* A. Gray, and ‘Ohi’a, *Metrosideros polymorpha* Gaud., causing higher incidence of tree limb breakage from their weight and wind blowdown (Warshauer *et al.*, 1983; La Rosa, 1984). Feral pigs feed on the fallen fruit, severely disturbing the soil and surrounding plants by their rooting, and disseminating the seeds in their excrement.

By the early 1980s, banana poka was considered the most serious threat to upper elevation forests of Hawaii, severely infesting 520 km$^2$ (Warshauer *et al.*, 1983). In 1982, the State of Hawaii appropriated funds to the Department of Forestry and Wildlife (DOFAW) for the evaluation of the potential for biological control of *P. mollissima*, marking the beginning of a concerted effort to control it.

This paper summarizes the results of foreign exploratory work from 1982 to 1996 in South America, describes the most promising insects that were studied, and identifies those having potential as biological control agents.

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## Materials and methods

*Passiflora* species of the subgenus *Tacsonia*, which include *P. mollissima*, occur throughout the Andes of South America, from 2000 to 3200 m, where they are cultivated for their fruits (Killip, 1938). *P. mollissima* and its related forms are typically associated with humans. Surveys included commercial stands, single plants or small stands around homes, and feral plants in natural or disturbed areas. Commercial sites frequently were exposed to regular applications of insecticide, which reduced their utility for insect collection.

Sampling of plants included visual inspection of foliage and dissection of flower buds, open flowers, fruit, roots, crowns and stems. Rearing and biological studies were conducted in laboratories in Venezuela (Causton *et al.*, 2000), Colombia and Hawaii. Host specificity testing was carried out in the Hawaii Volcanoes National Park (HVNP) insect quarantine facility. Voucher specimens were submitted to the USDA ARS Systematics Entomology Laboratory (SEL) in Beltsville, MD, as the definitive taxonomic authority. Many specimens came back as 'unidentifiable species', but a few new species descriptions were obtained. Specimens were retained at the USDA FS Institute of Pacific Island Forestry in Hilo, HI.

## Results

Species of the subgenus *Tacsonia* most commonly observed above 2200 m were *P. mollissima* (curuba, tumbo, taxo) and *P. mixta* L. Other species were only rarely observed, for example, *P. manicata* L., *P. pinnatistipula* Cav. Below 2200 m, *Tacsonia* species were replaced primarily by *P. edulis* Sims (i.e. maracuyá or passionfruit), *P. ligularis* Juss (granadilla amarilla) and *P. quadrangularis* L. (badea). We included *P. edulis* in our surveys due to concern over agent crossover to Hawaiian passionfruit, although its acreage and economic importance is small (Martin, 1994).

Table 1 summarizes the species of insects encountered. Where possible, the findings reported by Pemberton (1989), Causton *et al.* (2000) and unpublished reports by Markin and Friesen are included. The primary insect species studied during the course of the Hawaiian programme are briefly described below.

### Lepidoptera

***Cyanotricha necyria* Felder (Notodontidae):** This was the first species to be pursued as a biological control agent due to its severe impact on commercial production in western Colombia and central Ecuador, where severe outbreaks frequently defoliated the plants (Casañas-Arango *et al.*, 1990). *C. necyria* was tested and released in 1988 (Markin and Nagata, 1989; Markin *et al.*, 1989), but failed to become established in Hawaii (Markin *et al.*, 1989; Campbell *et al.*, 1993).

***Pyrausta perelegans* Hampson (Pyralidae=Crambidae):** This bud-feeder was widespread in Colombia, Ecuador, Peru and Venezuela. In Colombia, *Pyr. perelegans* was considered to be of major economic impact (Rojas and Chacón, 1983). The biology of *Pyr. perelegans* is discussed by Rojas and Chacón (1982). *Pyr. perelegans* was released in 1990 and is now established in Hawaii. However, populations have remained low and impact is negligible (Campbell *et al.*, 1993; Markin and Nagata, 2000).

***Acrocercops* nr. *pylonias* Meyrick (Gracillariidae):** This leaf-miner was commonly observed attacking *P. mollissima* and *P. mixta* in Colombia and northern Ecuador and possibly in Venezuela. In Colombia, damage due to *A.* nr. *pylonias* was incidental at all sites except for one near Pasto, where the leaf-miner was the dominant pest (Hugo Calvache, personal communication, December 1988). Late instar larvae form a distinct blotch that can cover several square centimeters of the upper leaf surface. Impact of the blotches was perceived small (Pemberton, 1989). This insect appeared promising and was scheduled for further field biology studies at the time the programme was cancelled in 1996.

***Odonna passiflorae* Clarke (Oecophoridae):** Larvae of this species bore the vines and root crowns of mature *P. mollissima*, that is, stems of 25–50 mm diameter. Multiple attacks in the root crown can kill the entire plant (Chacon and de Hernandez, 1981). Unfortunately, Colombia was the only country where we found *O. passiflorae*, and collecting the insect was difficult. One stand of *P. mollissima* was discovered near Lake La Cocha that suffered consistent losses of vines attributed to this pest species, but the planting was destroyed before extensive studies could be concluded.

**Heliconiidae: *Agraulis vanillae* L., *Dione glycera* C&R Felder and *D. juno* Cramer:** All three species were commonly observed at many of the survey sites and were polyphagous among *Passiflora* species, including *P. edulis* (Table 1). *D. juno*, a gregarious species of typically 20–50 larvae per cluster, was capable of denuding entire plants, while foliar damage due to *Agr. vanillae* was only sometimes significant, primarily on very young vines. *D. glycera* was widely distributed, but larval density was always very low and feeding damage negligible. *Agr. vanillae*, *D. juno* and *D. glycera* were particularly susceptible to nuclear polyhedrosis viruses (NPVs). *Agr. vanillae* is already present in Hawaii, but has had no discernible impact on *P. mollissima*.

***Josia fluonia* Druce (Notodontidae):** Larvae of this moth were observed feeding on foliage of *P. mollissima* only in central and northern Ecuador. The moths are day-flying. Larval density was low, that is, usually several larvae per plant, and feeding damage was typically light. *J. fluonia* was tested and cleared for release in 1996, but the programme was terminated before releases were made.

***Josia ligata* group (Notodontidae):** Larvae were observed feeding on foliage of *P. mollissima* and *P. mani-*

**Table 1.** List of insects observed feeding on *Passiflora* species in the Andes of Bolivia, Chile (Ch), Colombia, Ecuador, Peru, and Venezuela, 1982–1994. Information includes their order, family, insect species, *Passiflora* host species, stage host tissue, country. Table includes unpublished and previously published findings, e.g., Causton *et al*. (2000), Pemberton (1989), from the Hawaiian biological control project.

| Order | Family | Insect Species | Passiflora host species | Stage-Host tissue | Country[a] |
|---|---|---|---|---|---|
| Coleoptera | Buprestidae? | Unidentified sp. | *P. mollissima* | larvae—live stem | V |
| | Cerambycidae | Unidentified sp. | *P. mollissima* | larvae—dead crown | C |
| | | near *Hebestola* sp. | *P. mollissima* | larvae—dead stems | V |
| | | near *Lepturges* sp. | *P. mollissima* | larvae—dead stems | V |
| | | *Trachyderes* sp. | *P. mollissima* | larvae—dead stems | V |
| | Chrysomelidae | Cassidinae (unident. sp.) | *P. mollissima* | adults—leaves | V |
| | | *Diabrotica* sp. | *P. mollissima* | adults—leaves | C, V |
| | | *Epitrix* sp. | *P. mixta* | adults—leaves | E |
| | | | *P. mollissima* | adults—leaves | P, V |
| | | | *P. pinnatistipula* | adults—leaves | P |
| | | *Lactica brevicolis* Jacoby | *P. mollissima* | adults—leaves, flowers | V |
| | | *Lactica* sp.(?) | *P. mollissima* | adults—leaves, flowers | P |
| | | | *P. mixta* | adults—leaves, flowers | E |
| | | *Paralactica* sp.(?) | *P. manicata* | adults—leaves, flowers | E |
| | | | *P. mollissima* | adults—leaves, flowers | E, V |
| | Curculionidae | *Brachyomus* sp. | *P. mollissima* | adults—leaves, terminals | V |
| | | *Compsus* sp. | *P. mollissima* | adults—leaves, terminals | V |
| | | *Cryptorhyncus cerdo* Fiedler | *P. mollissima* | adults—leaves, terminals | V |
| | | *Exorides* ?*lajoyei* Bovie | *P. mollissima* | adults—leaves, terminals | V |
| | | *E.* ?*corrugatus* Marshall | *P. mollissima* | adults—leaves, terminals | V |
| | | *Pandeletius ?andeanus* Howden | *P. mollissima* | adults—leaves, terminals | V |
| | Elateridae | Unidentified sp. | *P. mollissima* | adults—? | V |
| | Lucanidae | Unidentified sp. | *P. mollissima* | adults—? | V |
| | Scarabidae | Unidentified sp. | *P. mollissima* | larvae—roots | B |
| | | Unidentified sp. | *P. mollissima* | adults—? | V |
| | Scolytidae | Unidentified sp. | *P. mollissima* | larvae, adults—stem, branches | V |
| Diptera | Drosophilidae | *Zapriothrica* nr *salebrosa* Wheeler | *P. mollissima* | larvae—flower buds | E, V, C |
| | | *Zapriothrica* nr *nudiseta* Wheeler | *P. mollissima* | larvae—flower buds | C |
| | | | *P. mixta* | larvae—flower buds | E, V |
| | | | *P. mollissima* x *P. exoniensis* | larvae—imm. fruit | V |
| | Lonchaeidae | *Dasiops caustonae* Norrbom and McAlpine | *P. manicata* | larvae—imm. fruit | E |
| | | | *P. mixta* | larvae—imm. fruit | V |
| | | | *P. mollissima* | larvae—imm. fruit | B, E, V |
| | | | *P. mollissima* x *P. exoniensis* | larvae—imm. fruit | V |
| | | *Dasiops curubae* Steyskal | *P. mollissima* | larvae—flower buds | B, E |
| | | *Dasiops gracilis* Norrbom and McAlpine | *P. edulis* | larvae—imm. fruit | V |
| | | *Dasiops inedulis* Steyskal | *P. edulis* | larvae—flower buds | B, V |
| | | *Dasiops* spp. (specimens unident.) | *P. mollissima* | larvae—flower buds | C |
| | | *Neosilba* sp. (poss *N. certa* Walker) | *P. mollissima* | larvae—imm. fruit | E |
| | Mycetophilidae | *Mycetophila* spp. | *P. mollissima* | larvae—flower buds | C, E, V |
| | | | *P. mixta* | larvae—flower buds | E |
| Hemiptera | Coreidae | *Leptoglossus* sp. | *P. mollissima* | adults, nymphs—fruit, stems | V |

*(continued on next page)*

**Table 1.** (Continued) List of insects observed feeding on *Passiflora* species in the Andes of Bolivia, Chile (Ch), Colombia, Ecuador, Peru, and Venezuela, 1982–1994. Information includes their order, family, insect species, *Passiflora* host species, stage host tissue, country. Table includes unpublished and previously published findings, e.g., Causton *et al*. (2000), Pemberton (1989), from the Hawaiian biological control project.

| Order | Family | Insect Species | Passiflora host species | Stage-Host tissue | Country[a] |
|---|---|---|---|---|---|
| | | Unidentified sp. | *P. mollissima* | adults—unknown | E |
| | Pentatomidae | Unidentified sp. | *P. mollissima* | adults—fruit? | V |
| | Pyrrhocoridae | Unidentified sp. | *P. mollissima* | adults—fruit? | V |
| | Tingidae | Unidentified sp. | *P. mollissima* | adults, nymphs—leaves | B |
| Hymenoptera | Apidae | *Trigona* sp. | *P. mollissima* | adults—flowers | V |
| Homoptera | Cicadellidae | Unidentified spp. (various) | *P. mollissima* | adults—leaves | V |
| | Cercopidae | Unidentified sp. | *P. mollissima* | adults—leaves | V |
| | Pseudococcidae | Unidentified sp. | *P. mollissima* | adults, immatures(?)—leaves | V |
| | Coccidae | Unidentified sp. | *P. mollissima* | adults, immatures(?)—leaves | V |
| Lepidoptera | Arctiidae | *Lophocampa* sp.? | *P. mollissima* | larvae—leaves | V |
| | | *Turuptiana neurophylla* | *P. mollissima* | larvae—leaves | C |
| | | *Turuptiana sanguinipectus(?)* Seitz | *P. mollissima* | larvae—leaves | E |
| | | Unidentified sp. | *P. mollissima* | larvae—leaves | V |
| | Coleophoridae | Unidentified sp. | *P. mollissima* | larvae—leaves | V |
| | Geometridae | Unidentified sp. | *P. mollissima* | larvae—leaves, flowers | B, E, V |
| | Gracillariidae | *Acrocercops* sp. near *pylonias* | *P. mollissima* | larvae—leaves | C, E, P |
| | | | *P. mixta* | larvae—leaves | E |
| | | Unidentified sp. | *P. mollissima* | larvae—leaves, fruits | V |
| | Heliconiidae | *Agraulis vanillae* L. | *P. mollissima* | larvae—leaves | B, C, E, P, V |
| | | | *P. edulis* | larvae—leaves | E, V |
| | | | *P. ligularis* | larvae—leaves | E, P |
| | | | *P. manicata* | larvae—leaves | E, P |
| | | *Dione glycera* C&R Felder | *P. alata* | larvae—leaves | V |
| | | | *P. edulis* | larvae—leaves | V |
| | | | *P. ligularis* | larvae—leaves | V |
| | | | *P. mollissima* | larvae—leaves | B, C, Ch, E, P, V |
| | | *Dione juno* Cramer | *P. edulis* | larvae—leaves | E, P, V |
| | | | *P. ligularis* | larvae—leaves | E |
| | | | *P. manicata* | larvae—leaves | E |
| | | | *P. mollissima* | larvae—leaves | V, E |
| | | *Euptoieta hegesia* Comstock | *P. mollissima* | larvae—leaves | V |
| | Noctuidae | *Copitarsia* sp. (?) | *P. mollissima* | larvae—leaves, flower buds, | E, V, P |
| | | *Copitarsia consueta* | *P. mollissima* | larvae—flowers | C |
| | Notodontidae[b] | *Cyanotricha necyria* Felder | *P. manicata* | larvae—leaves | E |
| | | | *P. mollissima* | larvae—leaves | C, E, P |
| | | *Josia fluonia* Druce [not released] | *P. mollissima* | larvae—leaves | E |
| | | *Josia ligata* group | *P. mollissima* | larvae—leaves | E |
| | | | *P. manicata* | larvae—leaves | E |
| | Oecophoridae | *Odonna passiflorae* Clarke | *P. mollissima* | larvae—stems | C |
| | Psychidae | Unidentified sp. | *P. mollissima* | larvae—leaves | B, V |
| | Pyralidae (= Crambidae) | *Pyrausta perelegans* Hampson | *P. mixta* | larvae—flower buds, fruit stem tips | E, P, V |

**Table 1.** (Continued) List of insects observed feeding on *Passiflora* species in the Andes of Bolivia, Chile (Ch), Colombia, Ecuador, Peru, and Venezuela, 1982–1994. Information includes their order, family, insect species, *Passiflora* host species, stage host tissue, country. Table includes unpublished and previously published findings, e.g., Causton *et al*. (2000), Pemberton (1989), from the Hawaiian biological control project.

| Order | Family | Insect Species | Passiflora host species | Stage-Host tissue | Country[a] |
|---|---|---|---|---|---|
| | | | *P. mollissima* | larvae—leaves, flower buds, stem tips | C, E, V, P |
| | Saturniidae | Unidentified sp. | *P. mollissima* | larvae—leaves | V |
| | Tortricidae | Unidentified sp. | *P. mollissima* | larvae—leaves, stems, stem tips | V |
| | | Unidentified sp. | *P. mollissima* | larvae—leaves | V |
| Lepidoptera | Unidentified | Unidentified sp. | *P. mollissima* | larvae—stems | V |
| Orthoptera | Acrididae | *Meridacris subaptera* | *P. mollissima* | adults—leaves | V |
| Thysanoptera | unidentified | Unidentified sp. | *P. mollissima* | adults, nymphs—unknown | V |

[a] B = Bolivia; Ch=Chile; C=Colombia; E=Ecuador; P=Peru; V=Venezuela
[b] For the family Notodontidae, we used the classification of Miller (1996).

*cata* only in central and northern Ecuador. Larvae of *J. ligata* were very similar to *J. fluonia* larvae in appearance, behaviour and feeding damage. However, *J. ligata* was found to be able to complete development on several *Passiflora* species, including *P. edulis*, dropping it from further consideration.

## Diptera

***Zapriothrica* nr. *salebrosa* Wheeler (Drosophilidae):** This flower bud-attacking fly was probably the most common and widely distributed insect found during our surveys and has long been recognized as a pest of *P. mollissima* (Chacon and Rojas, 1984; A.D. Casañas, 1984, unpublished results); it was observed in Colombia, Ecuador, Peru and Venezuela. However, Casañas-Arango *et al.* (1996) and Causton *et al.* (2000) mention another similar species, *Z.* nr. *nudiseta*, attacking only *P. mollissima* in Colombia, suggesting that taxonomic review of this group may be necessary. Pemberton (1989) identified *Z.* nr. *salebrosa* as a candidate agent and preliminary studies were conducted in the field in Colombia. The biology of *Zapriothrica* sp. is discussed by Casañas-Arango *et al.* (1996). The release of *Pyr. perelegans*, another bud-feeder, and studies of a fruit-attacking fly *Dasiops caustonae* Norrbom and McAlpine (Diptera; Lonchaeidae) lead to putting studies of *Z.* nr. *salebrosa* on hold.

***Dasiops* species (Lonchaeidae):** Species of the genus *Dasiops* have long been recognized as major pests of cultivated species of *Passiflora* (Posada *et al.*, 1976; Chacon and Rojas, 1984). Lonchaeid larvae were regularly encountered in flower buds or in the developing fruit in all of the countries surveyed except Chile (Table 1), often causing significant losses of fruit bodies. Field identification of the species proved to be impossible. A taxonomic review of *Dasiops* species associated with *Passiflora* described three of the five species we collected from *P. mollissima* as capable of attacking *P. edulis*, that is *D. curubae*, *D. gracilis* and *D. inedulis* (Norrbom and McAlpine, 1997). A newly described species, *D. caustonae* Norrbom and McAlpine, appeared to be confined to *Passiflora* species in the subgenus *Tacsonia*, except for *P. manicata*, and was the only insect found attacking the developing fruit. Its biology is discussed by Causton and Rangel (2002). Attempts to colonize *Dasiops* species in quarantine in Hawaii failed due to our inability to induce mating, although oviposition of sterile eggs readily occurred.

***Mycetophila* (Mycetophilidae):** At least two species of these fungus gnats were observed attacking flower buds in Northern Ecuador , Colombia and Western Venezuela; bud loss was often very significant. Specimens of adults could only be determined to the genus level (Gagné, personal communication, 1995). Multiple larvae were found in each infested bud, with up to 17 in one bud (Causton *et al.*, 2000). The larvae were very sensitive to disturbance and/or dehydration, as healthy larvae within dissected buds usually died shortly after inspection.

## Coleoptera

**Longhorned Beetles (Cerambycidae):** Cerambycids were collected from mature vines of *P. mollissima*, that is, older than 8 years, at two locations. Larvae of an unidentified species, 1–2 cm long, were recovered from root crowns of dead vines in the Lake La Cocha area in Colombia. At least three species of cerambycids, near *Hebestola* sp., near *Lepturges* sp. and *Trachyderes* sp., were recovered from dead or dying branches of vines

in Venezuela (Table 1). Further investigation will be necessary to confirm their identities and feeding type, that is, as primary or secondary.

**Leaf Beetles (Chrysomelidae):** Adults of several species of leaf beetles were observed feeding on foliage and/or blooms of several *Passiflora* species in Colombia, Ecuador, Peru and Venezuela, but no eggs or larvae were recovered in the field and breeding colonies could not be established in the laboratory. Adults of *Diabrotica* sp. and *Epitrix* sp. were quite common in Venezuela, but were observed to be polyphagous (Causton *et al.*, 2000).

## Discussion

Except for the fungal pathogen, *Septoria passiflorae* Syd. released in 1996, no new insect agents have been released since *Pyr. perelegans* in 1990. However, progress was made in identifying several promising candidates and eliminating others. Below is a ranking and brief justification of the agents we consider most promising for future work: (1) *Z.* nr. s*alebrosa*/*Z.* nr *nudiseta* because of their potential impact on reproduction of *P. mollissima*. Their biology and colonization techniques have already been determined, although species identities and distinctions need to be clarified; (2) *O. passiflorae* was capable of killing mature plants. Aspects of its biology and a local collaborator for collection of the species are known, which could facilitate initiation of biological studies; (3) *D. caustonae* and (4) *Mycetophila* sp. due to their potential to significantly impact reproduction of *P. mollissima*. Before studies may progress, the problem of establishing reproducing colonies in captivity needs to be solved. Host testing in the insects' country of origin could bypass this problem; and (5) the unidentified lepidopteran stem borer from Venezuela (Causton *et al.*, 2000). Like *O. passiflorae*, this species is recognized as having potential for significantly increasing plant mortality in Hawaii. However, this insect species was only observed once throughout the duration of the 4-year Venezuelan study. Confirmation of its identity remains first priority. Other candidates of lower priority but of potential interest include the chrysomelid beetles from northern Ecuador and cerambycid beetles from Colombia and Venezuela. As a note, although *C. necyria* and *Pyr. perelegans* were not successful in Hawaii, they may be well suited for different environments.

A third biological control agent, the fungal pathogen *S. passiflorae*, was also identified during the Hawaiian programme, tested and released in 1996 (Trujillo, 2001). The pathogen is now established through parts of the range of *P. mollissima* in Hawaii and is credited with giving substantial reduction in biomass by causing early defoliation in certain areas (Trujillo, 2001, 2004). However, in parts of the range, the pathogen is ineffective or is no longer giving adequate control (Markin, personal communication, May 2006).

*P. mollissima* remains an invasive exotic species in Hawaii and in other parts of the world. If, in the future others should decide to attempt biocontrol of *P. mollissima*, we hope that this summary of our survey results will be of value.

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# A cooperative research model – biological control of *Parkinsonia aculeata* and Landcare groups in northern Australia

**V.J. Galea**[1]

*Summary*

Parkinsonia, *Parkinsonia aculeata* L., is a woody shrub, which is classed as a weed of national significance in Australia. It is considered a major threat to both managed and natural ecosystems. Research into the cause of a dieback disorder in Parkinsonia occurring at locations across northern Australia has identified a range of fungal organisms to be associated with affected plants. Currently, these are being evaluated in conventional field trials at locations in north Queensland and the Northern Territory. A cooperative research model has been developed to allow regional Landcare groups to participate in this research programme. This standardized model for medium-scale trials will enable Landcare groups to establish, monitor and evaluate the performance of a range of potential biological control agents under local conditions. The development of a research kit is a key element of this programme. The kit will include equipment needed to establish the trial and the fungal agents to be evaluated. An instruction manual will outline the procedures required to select an appropriate trial site and provide instructions on inoculation, data collection and ongoing maintenance of the trial. This cooperative approach will both engage and enable Landcare groups in the development of solutions for their regions.

***Keywords***: cooperation, research, Parkinsonia.

## Introduction

Parkinsonia, *Parkinsonia aculeata* L., is a woody shrub, which is classed as a Weed of National Significance (WoNS) in Australia. It is considered a major threat to both managed and natural ecosystems (Deveze *et al.*, 2004). Parkinsonia currently infests approximately 1 million ha of land, mainly along watercourses throughout northern Australia. Parkinsonia severely degrades the economic and environmental value of land that it invades (Deveze *et al.*, 2004). The spread of this species threatens biodiversity, the health of river systems and wetland areas and the productivity of pastoral enterprises (personal observation). It forms dense impenetrable thickets, which reduce native fauna habitat and impede mustering activity and stock access to water (Diplock *et al.*, 2006). It replaces native and pasture species and reduces the carrying capacity and productivity of pastoral land.

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Biological control of Parkinsonia is a key element of strategic management (van Klinken, 2006). While chemical treatment of minor infestations is often effective, the establishment of biological control agents may promise to give lasting control.

Parkinsonia dieback is a disorder, which causes Parkinsonia plants to dieback from the tips; the leaves droop, turn brown, but remain attached to the plant, which eventually dies. Dieback progresses through Parkinsonia populations as a front and kills both adult and juvenile plants (Diplock *et al.*, 2006). The disease appears to be naturally occurring in the Northern Territory, Western Australia and Central Queensland. There appear to be four key fungal organisms associated with Parkinsonia dieback, which are either native fungal species, or species that are naturalized and now widespread (Diplock *et al.*, 2006). Field observations and historical reports suggest that this dieback disorder has the potential to be harnessed as a self-replicating and potentially, self-dispersing biological control tool. A better understanding of the way in which the fungal species infect the plant causing disease and death, and the related factors that contribute to disease develop-

ment (i.e. environmental stresses, plant density and age, impact of management practices, fire, etc.) could lead to the development of an integrated management programme for Parkinsonia involving the use of these potential biological control agents as a key element.

Postgraduate research (Diplock *et al.*, 2006) carried out by the University of Queensland (UQ) into the cause of the dieback disorder in Parkinsonia has identified a range of fungal organisms to be associated with affected plants. A culture bank developed for this project contains over 200 isolates taken from field affected Parkinsonia plants collected from across northern Australia. Within this collection, four key genera (*Fusarium*, *Lasiodiploidia*, *Phoma* and *Fusicoccum*) have been identified as being widely distributed and are being treated as organisms of interest. Currently, these four genera are being evaluated in conventional field trials at locations in north Queensland and the Northern Territory and in laboratory and glasshouse studies at UQ.

## Objectives

- To build on previous and ongoing university research and to serve as a model for a national approach for the cooperative development of knowledge and the creation of regional capacity for pathogen-based woody weed biocontrol programmes;
- To develop a standardized field research model including training and resources to build the capacity of stakeholders to undertake Parkinsonia dieback trials;
- To collect data that will contribute to the long-term research into Parkinsonia biological control;
- To communicate the outcomes of the project to the community and other researchers; and
- To develop appropriate delivery technologies for Parkinsonia dieback pathogens that will be appropriate for on-ground managers.

# Methodology

This project involves trials being set up within two National Parkinsonia Management Zones. Thirty sites are to be set up in total with resources available to set up more sites if required. Fifteen sites will be established in Zone A (Victoria River District) and 15 in Zone B (Barkly region). This project will develop a standardized model for medium-scale trials (based on methods currently used for a PhD programme on Parkinsonia dieback) which will enable Landcare groups to establish, monitor and evaluate the performance of a range of potential biological control agents under local conditions. A support and communication officer will be appointed to develop materials and support stakeholders. The current inoculation method involves the introduction of a formulated inoculum pellet into the stem of Parkinsonia trees. Protocols for site and tree selection, assessment of critical parameters for tree health, inoculation and site mapping have been established, but may be modified after consultation with client groups.

There are seven key elements to this programme:

1. **Development of an instruction manual**. An instruction manual will be prepared, outlining the procedures required to select an appropriate trial site and outline the procedure for selecting appropriate trees, and performing pre- and postinoculation assessment for size and vigour. The manual will also provide instructions on how to inoculate trees, postinoculation data collection (both quantitative and qualitative) and ongoing maintenance of the trial.
2. **Preparation of project kits**. Project kits will be prepared for each of the cooperator teams. These will include the Instruction Manual along with some of the materials required to establish the trial, that is, the fungal inoculum in a stable form, tree tags, a water squirt bottle, PVC tape to seal the tree wound, flexible tape measure for stem circumference measurement, and additional data sheets. The kits will be prepared and mailed out as required. Additional inoculum and tags can be supplied for groups wishing to establish more than one trial site.
3. **Running instructional workshops**. Two training workshops/field days will be conducted to outline the procedures and rationale for this work as well as assessment and data management procedures. These workshops will also provide an opportunity to gain information from land and landscape managers about Parkinsonia and their expectations from the cooperative research programme.
4. **Ongoing support for trials**. Support for trials will be available through e-mail and telephone communication. A programme coordinator and project support officer will be available to visit trial sites in each region.
5. **Use of data**. Data collected by cooperators will be used internally in property management planning. Additionally, data will be shared among groups and also shared with the overall project coordinator to contribute to the knowledge base on this biocontrol system, assisting UQ research. The type of data to be collected includes plant vigour before and after inoculation with a pathogen, stem circumference and symptoms of plant disease such as loss of stem integrity, presence of basal stem lesions, or other related symptoms. Collection of (digital) photographic evidence will also be supported.
6. **Reporting and communications**. Outcomes from the trials will be shared by a biannual newsletter to be distributed by electronic means. Additionally, outcomes will be presented at appropriate symposia and conferences and published in appropriate newsletters (Barkly Beef, Weed All About it, Barkly Land Care Association and Victoria River

District Conservation Association newsletter, Network Notes) to ensure maximum possible sharing of information.

7. **Extension of new knowledge from laboratory research**. As new biocontrol isolates or inoculation techniques become available from the associated UQ projects, these will be communicated to the cooperator groups, and where possible made available for field testing.

# Identified collaborators

Strong support for this project has been demonstrated by existing collaborators and new potential collaborators. Victoria River District Conservation Association (VRDCA) and Roper River Landcare Group (RRLG) are currently supporting the project with preliminary sites already set up. Members of the VRDCA and the RRLG are currently being trained in inoculation techniques and plant vigour assessment.

The Weed Management Section of the Department of Natural Resources, Environment and the Arts (NRETA) will also support the project by providing technical advice and assistance to all stakeholders, attending workshops and participating in setting up and monitoring trial sites.

Aboriginal Ranger Groups (Muru-warinyi Ankkul Rangers) are keen to participate in the project to increase skills, build capacity among the groups and improve networks between stakeholders.

The Department of Primary Industry, Fisheries and Mines (DPIFM) and the Northern Territory Cattlemen's Association (NTCA) also support this project as Parkinsonia impacts on pastoral operations and the pastoral industry within the Northern Territory. Both support research into effective biological control agents for use as a tool.

# Justification

Current research on Parkinsonia dieback being conducted by UQ has over the past 2 years attracted significant attention among landscape managers from Parkinsonia-affected regions and researchers involved in woody weed management. Through the involvement of VRDCA, RRLCG, NRETA Weed Management and Aboriginal ranger groups, UQ will deliver a project that achieves a strong community-based landcare movement through improving communication and collaboration between land managers across a range of tenures. This project involves local stakeholders, that is, Landcare groups, aboriginal rangers, NRETA weeds officers and UQ creating a cooperative collaborative approach across regions and state boundaries. The partnerships created will improve regional planning. Parkinsonia biological control is still in development. Through the involvement of local stakeholders, it will be easier to achieve research goals. Training workshops and the production of kits will increase the capacity of land managers to undertake Parkinsonia dieback trials. This project will raise awareness of adoption of integrated weed management practices.

## Significance of expected outcomes

- A partnership between Government agencies, regional community leaders, indigenous ranger groups and pastoralists will promote implementation of VRD components of the Katherine Regional Weed Management Plan (M. Kassman, personal communication, 2006). This ensures that all stakeholders have a voice in the execution of strategic weed management, supporting the VRDCA model of holistic management in the spheres of production, community and conservation, and the Integrated Natural Resource Management Plan (Northern Territory) vision of fostering strong local contribution to natural resource management.
- Participation in, and visual confirmation of, the efficacy of sustained weed control programmes will provide an impetus for further long-term planning and investment from stakeholders in the regions.
- This project directly contributes to the goal of sustainable natural resources and community capacity building by integrating planning, training and action.

# Concluding comments

Experience from previously established pilot trials with the Barkly Landcare and Conservation Association (BCLA) and the Roper River Landcare Group (RRLG) have indicated both a high level of willingness to engage in cooperative research and accelerated outcomes through the sharing of knowledge and experience held by such groups. This approach has proven to be an excellent way of guiding research to ensure that outcomes are more appropriately aligned with the needs of landholders and landscape managers. Adoption of outcomes will be greater for cooperatively developed management strategies; furthermore, this approach may ensure that localized environmental and operational conditions are considered.

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# A global view of the future for biological control of gorse, *Ulex europaeus* L.

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*Summary*

Gorse (*Ulex europaeus* L.) has become naturalized in at least 50 countries outside its native range, from the high elevation tropics to the subantarctic islands and Scandinavia. Its habit, adaptability and ability to colonize disturbed ground makes it one of the world's most invasive temperate weeds. It is 80 years since New Zealand first initiated research into biological control for gorse. This paper briefly reviews the progress made worldwide since then, and examines future opportunities for biological control of this weed. The range of available agents is now known, and this list is critically assessed. Ten organisms have been released variously in six countries and islands and their performance is reviewed. In most cases, agent populations have been regulated either from 'top-down' or 'bottom-up', and there is no evidence anywhere of consistent outbreaks that could cause significant reduction in existing gorse populations in the medium term. Habitat disturbance and seedling competition are important drivers of gorse population dynamics. Existing agents may yet have long-term impact through sublethal effects on maximum plant age, another key factor in gorse population dynamics. Along with habitat manipulation, seed-feeding insects may yet play a long-term role in reducing seed banks below critical levels for replacement in some populations. In the short term, progress will rely on rational and integrated weed management practices, exploiting biological control where possible.

***Keywords***: integrated weed management, population dynamics, modelling.

## Introduction

Gorse, *Ulex europaeus* L. (Fabaceae), is a thorny shrub native to the temperate Atlantic coast of Europe and the British Isles including Ireland. It has become naturalized elsewhere in Europe, North Africa and the Middle East. In other parts of the world, gorse has proven to be an aggressive invader, forming impenetrable, largely monotypic stands that reduce access of grazing animals to fodder, modify native ecosystems and ecosystem processes, and outcompete trees in developing forests. It has now been recorded in more than 50 countries and islands, and is considered to be a major weed in New Zealand (Hill *et al.*, 2000), the USA (Markin *et al.*, 1995, 1996), Chile (Norambuena *et al.*, 2007) and Australia (Ireson *et al.*, 2006).

Gorse is tolerant of a wide range of conditions, but in temperate latitudes, it is limited altitudinally by cold temperatures. This has not stopped it becoming established in the montane regions of tropical Hawaii, Sri Lanka and Costa Rica, and it grows in Scandinavia, on St. Helena, on some subantarctic islands, and in coastal NE USA, where maritime influences moderate the climate. New Zealand is one of the few places where gorse has largely achieved its maximum distribution.

Land managers on intensively managed land with adequate economic returns have many excellent alternatives for managing gorse. However, biological control may provide the only option for limiting the effects

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of gorse on land that provides low economic returns, land that is managed for biodiversity values, or where the infestation is simply too difficult to manage.

It is now more than 80 years since research into the biological control of gorse in New Zealand was first commissioned (Zwölfer, 1963), and the first biological control agent, *Exapion ulicis* Forster (Brentidae), was first released on Maui, HI, in 1926 (Markin *et al.*, 1996). Ten biological control agents have now been released as classical biological control agents in six countries or regions: two seed-feeding insects and eight organisms that attack green stems (Table 1). It is now time to examine the state of gorse biological control worldwide, and its future role in gorse management. The purpose of this paper is to:

- evaluate the effects to date of biocontrol agents released worldwide;
- examine the options for the development of new biocontrol agents; and
- explore how biological control might interact with other management techniques.

## Current role of biocontrol agents in gorse management worldwide

### *Exapion ulicis* (Forster)

The gorse seed weevil, *E. ulicis*, is now widely established in New Zealand, Australia, the USA (the West Coast and Hawaii) and Chile (Table 1). In New Zealand, the weevil only attacks pods in spring, whereas gorse sets seed in both spring and autumn. Where the bulk of annual seed production is in autumn, almost all seeds escape attack. Where the bulk of seed production is in the spring, infestation rates are low because of the abundance of the food source available to the weevil (Hill *et al.*, 1991a). Either way, this results in approximately 65% of the annual seed crop escaping attack (Cowley, 1983). As in New Zealand, Davies (2006) showed that larvae feed on seeds produced in spring and summer in Australia (Tasmania), and were not present during a second period of seed production during autumn and winter. He found that damage to gorse seed ranged from 12.4% to 55.4% and varied annually within and between sites. In Chile, *E. ulicis* is able to reduce gorse seed production and dispersal (Norambuena and Piper, 2000) but has had only limited impact upon gorse invasiveness to date. The same is true in the western United States (Markin *et al.*, 1995) and on the islands of Maui (1953) and Hawaii (1984) (Markin and Yoshioka, 1998).

### *Cydia succedana* (Denis and Schiffermüller)

With the realization that *E. ulicis* alone was unlikely to reduce seed production to low levels at most sites, a strong focus of research and development since 1982 has been the search for new control agents that could augment the activity of the univoltine weevil in spring, and also reduce the autumn seed crop that currently escapes attack. *C. succedana* was chosen for release in New Zealand to fill this role (Table 1) as it has two generations per year in its home range; one in spring on pods of *U. europaeus* and another on pods of late summer- and autumn-flowering gorse species. The moth is now abundant throughout gorse-infested areas of New Zealand. Gourlay *et al.* (2004) used insecticide exclusion to show that *C. succedana* augmented control gorse seed predation by *E. ulicis* and recorded an overall 81% loss in spring seed production at one site. However, the predicted reduction in autumn seed production has not occurred. There appears to be lack of synchrony between the emergence of moths and the peak occurrence of *U. europaeus* pods in autumn, and infestation rates rarely exceed 10%. As seed set in autumn forms the bulk of seed production in warmer parts of New Zealand, adequate control of seed production has not yet been achieved.

### *Tetranychus lintearius* Dufour

Populations of the mite *T. lintearius* (Tetranychidae) initially increased rapidly in the countries where it was released (Table 1). Colonies formed massive webs over gorse and caused severe bronzing of the foliage. However, in New Zealand and Australia, populations have declined at all sites after the initial increase, and although localized outbreaks still occur, widespread outbreaks are now rare. Mite populations in New Zealand appear to be regulated by the predators *Stethorus bifidus* Kapur (Coccinellidae) and *Phytoseiulus persimilis* (Athias-Henriot) (Phytoseiidae).

In Australia (Tasmania), Davies *et al.* (2007) showed that the presence of mite colonies on gorse bushes over a period of 2.5 years from the time of release reduced foliage dry weight by around 36%. However, predation of *T. lintearius* colonies by *Stethorus* sp. and *P. persimilis* is widespread in Australia (Ireson *et al.*, 2003) and probably a key factor in restricting the impact of the mite. *P. persimilis* has been associated with the destruction of entire colonies in both Tasmania and Victoria (Ireson *et al.*, 2003) as well as in Oregon, USA (Pratt *et al.*, 2003).

Regulation by predators does not yet appear to be as severe in Hawaii and Chile. The mite has been abundant in Hawaii since 2000. Chemical exclusion from paired bushes ($n$ = 10) indicated that mite feeding reduced gorse shoot elongation by 37% and flowering by 82% (G. Markin, unpublished data). Similar effects have been measured in unpublished New Zealand studies, but only where infestation is persistent. Predation usually precludes such long-term damage. In Chile, mite populations have grown strongly at 90% of release sites, especially in relatively dry areas, despite predation

**Table 1.** Status of biological control agents released worldwide against gorse (N = not established, R = recovered, not yet established, E = established, HI = Hawaii, WC = west coast).

| Control agent | Taxonomic group | Target country | Date of release | Status (N, R, E) | Comments | References |
|---|---|---|---|---|---|---|
| *Exapion ulicis* (Forster) | Brentidae | New Zealand | 1931 | E | Larvae attack seeds in pods | Hill *et al.*, 1991a |
| | | USA, HI | 1926 | N | | |
| | | | 1958, 1984 | E | First on Maui and then Hawaii | Markin *et al.*, 1996; Markin and Yoshioka, 1998 |
| | | USA, WC | 1953 | E | | Davis, 1959; Markin *et al.*, 1995 |
| | | Australia | 1939 | E | Established in TAS, VIC, NSW and SA | Ireson *et al.*, 2006 |
| | | Chile | 1976 | E | Seed production and dispersal decreased | Norambuena *et al.*, 1986 |
| *Apion* sp. | | USA, HI | 1958 | N | Seed in pods | |
| *Eutrichapion scutellare* Kirby) | Brentidae | USA, HI | 1961 | N | Forms galls | Markin *et al.*, 1995 |
| *Tetranychus lintearius* Dufour | Acari | New Zealand | 1989 | E | Extracts mesophyll | Hill *et al.*, 1991b, 1993 |
| | | USA, HI | 1995 | E | | |
| | | USA, WC | 1994 | E | | Pratt *et al.*, 2003 |
| | | Australia | 1998 | E | Established in TAS, VIC, NSW, SA and WA | Ireson *et al.*, 2003 |
| | | Chile | 1997 | E | | Norambuena *et al.*, 2007 |
| | | | 2006 | R | Released and recovered at 50 new points between 41º53' and 43ºS | H. Norambuena, personal communication, 2006 |
| | | St. Helena | 1995 | E | | S.V. Fowler, personal communication, 2006 |
| *Scythris grandipennis* (Haworth) | Scythrididae | New Zealand | 1990 | N | Larvae defoliate from solitary web | Hill *et al.*, 2000 |
| *Agonopterix ulicetella* (Stainton) | Oecophoridae | New Zealand | 1990 | E | Larvae defoliate from solitary web | Hill *et al.*, 1995 |
| | | USA, HI | 1988 | E | | Markin *et al.*, 1996 |
| | | USA WC | | | | Markin *et al.*, 1995 |
| | | Australia | | | Release approved; population ex NZ currently in quarantine F31 | |
| | | Chile | 1997 | R | Recovered the following year but establishment not confirmed; good damage potential in confinement | Norambuena *et al.*, 2004 |
| | | | 2006 | R | Released at 40 new points between 41º53' and 43ºS | H. Norambuena, personal communication, 2006 |
| *Sericothrips staphylinus* Haliday | Thripidae | New Zealand | 1990 | E | Extracts mesophyll | Hill *et al.*, 2001 |
| | | USA, HI | 1990 | E | | Markin *et al.*, 1996; Hill *et al.*, 2001 |
| | | Australia | 2001 | E | Established in TAS and VIC, establishment in NSW and SA not confirmed | Ireson *et al.*, 2006 |

**Table 1.** (Continued) Status of biological control agents released worldwide against gorse (N = not established, R = recovered, not yet established, E = established, HI = Hawaii, WC = west coast).

| Control agent | Taxonomic group | Target country | Date of release | Status (N, R, E) | Comments | References |
|---|---|---|---|---|---|---|
| *Cydia succedana* (Denis and Schiffermüller) | Tortricidae | New Zealand | 1992 | E | Larvae feed on seeds in pods | Hill and Gourlay, 2002 |
| *Pempelia genistella* Duponchel | Pyralidae | New Zealand | 1996 | E | Larvae defoliate from communal web | Hill *et al.*, 2000 |
| | | USA, HI | 1996 | R | | Markin *et al.*, 1996, 2002 |
| *Uromyces pisi (DC.) Otth.* f. sp. *europaei* Wilson and Henderson | Uredinales | USA, HI | 2000 | R | Single pustule after 2 years, but not seen since | Culliney *et al.*, 2003 |

by *Oligota centralis* Sharp (Staphylinidae). The stress of mite attack has slowed plant growth, flowering has become almost totally disrupted, and occasional seedlings have been killed. The mite is now being distributed widely in Chile (Norambuena *et al.*, 2007), but in Hawaii, populations have declined recently, and it is feared that regulation by a phytoseiid new to this montane region is underway.

## *Sericothrips staphylinus* Haliday

*Sericothrips staphylinus* (Thripidae) is now widespread in Hawaii and parts of New Zealand, and has now become established in Australia (Table 1). No field studies on the efficacy of this species under field conditions have been conducted. However, a glasshouse study in Australia (Tasmania) showed that a combination of feeding by gorse thrips, ryegrass competition and simulated grazing resulted in a gorse seedling mortality of 93% compared with no mortality in the untreated control, and reduced shoot dry weight of seedlings (Davies *et al.*, 2005). This experiment indicates the potential of *S. staphylinus* in an integrated control programme if field populations are eventually able to increase to sufficient densities. As yet no visible damage attributable to *S. staphylinus* has been observed at Tasmanian field sites up to 6 years after release. The maximum estimated field population density of juveniles and adults has been ca. 1.5 thrips $cm^{-1}$ of new growth. In comparison, population densities of ca. 7 thrips $cm^{-1}$ of new growth have been measured in glasshouse cultures, from plants on which severe feeding damage was recorded (J. Ireson, unpublished data).

## *Agonopterix ulicetella* (Stainton)

Despite heavy parasitism, larvae of the oecophorid moth *A. ulicetella* destroy a high proportion of gorse shoot tips in the Hawaiian infestation each spring. However, the control agent is univoltine, and the period of damage is short. Gorse plants appear to compensate for the loss within the growing season by initiating new shoots, and the effect on growth rate or biomass accumulation per plant is not known. This is also true in New Zealand, where outbreak populations have begun to appear in some sites since 2005, 15 years after the moth was first released. While the moth produced substantial damage to gorse shoots enclosed within a fine mesh sleeve in Chile (Norambuena *et al.*, 2004), field establishment has not been confirmed.

## *Pempelia genistella* Duponchel

A small population of this pyralid moth was observed for several years following its release in Hawaii. No larvae have been detected for some years. Recent applications of herbicides and fire destroyed the original release sites, and the persistence of this species is in doubt. In New Zealand, *P. genistella* has established well at only a limited number of sites near Christchurch, despite widespread releases nationwide (Table 1). The reasons for this are not known, and its future role in gorse management remains uncertain.

## *Uromyces pisi* (DC.) Otth. f. sp. *europaei* Wilson and Henderson

In 2002 a single pustule of this rust was detected in Hawaii near where it was released 2 years previously. It was a new infection locus, and urediniospores were being produced. No additional rust pustules have been found since, so its continued establishment must be in doubt.

## Bioherbicides for gorse management

In addition to the classical biocontrol agents, two pathogens are being formulated in New Zealand as bioherbicides of gorse. It has proven difficult to formulate *Fusarium tumidum* Sherb. in a way that produces consistent damage to gorse foliage, but Bourdôt *et al.* (2006) recently showed that both *F. tumidum* and *Chondrostereum purpureum* (Pers.:Fr.) Pouzar may have potential as mycoherbicides for gorse regenerating after mowing or trimming.

## Options for the development of new agents

Surveys of potential biocontrol agents for gorse have been conducted in the native range of gorse over a long period. Zwölfer (1963) reported on European literature records, and CABI staff undertook surveys of the fauna in France. Zwölfer's report did not specifically state the time of year of the surveys, but they were generally considered to have been carried out in spring (Sheppard, 2004). R.L. Hill (1982, unpublished results) completed a detailed study of the seasonality of gorse insects in southern England. O'Donnell (1986) surveyed NW Spain and Portugal in spring, listing brief site descriptions and the agent species identified. Other less formal surveys by authors of this paper explored the fauna of gorse as far south as Sintra, NW of Lisbon in spring (R. Hill and G. Markin, unpublished data). Sheppard (2004) combined the information from all of these sources and this summary is now considered to be the definitive list of invertebrates that have potential as control agents for gorse.

The rate at which gorse spreads into new habitats is likely to be strongly related to the amount of seed produced, and reducing the annual seed crop using seed-feeding agents may slow spread and give land managers opportunities to protect vulnerable habitats. *E. ulicis* and *C. succedana* singly and in combination reduce the annual seed crop of gorse in New Zealand (Hill *et al.*, 2004) but not sufficiently to cause population decline (although it is possible that the long-lived seed bank masks such an outcome, and these effects may become apparent in the future). For New Zealand, the solution lies in finding control agents that attack pods formed in autumn. Several *Apion* species are known to take this role (Zwölfer, 1963). *Cydia internana* (Guerin) also fills this role, but this is a rare species in the UK (Hill, 1982). The introduction of *C. succedana* to regions outside New Zealand remains an option, but its unpredicted appearance on hosts related to gorse following release in New Zealand demands caution and further research. Such research is in progress (Fowler *et al.*, 2004).

The introduction of additional seed-feeding agents would also be useful for Australia, especially as there are many sites where gorse sets seed only once per year. The planned introduction of *C. succedana* is now unlikely (Ireson *et al.*, 2006) although it may still be considered once additional data on its true host range and the level of damage caused to alternative hosts is considered. Ultimately, the introduction of an additional seed feeder to Australia may depend on the discovery of host-specific biotypes of known species from Europe, although no such autumn seed feeders were found in recent surveys (Sheppard, 2004). Foliage-feeding agents may also reduce annual seed production by reducing plant vigour, but as yet we know little about the strength of this effect.

The primary aim of the field surveys conducted in Europe during 2003 (Sheppard, 2004) was to identify agents capable of reducing seed production in autumn. There were only low levels of pod production during the period of the survey, and losses of *Ulex* spp. seed to pod moths (*Cydia* spp.) and *E. ulicis* were considered minor. There was also no evidence that there were any other autumn-specific seed-feeding agents active during this period (Sheppard, 2004).

Surveys for root-feeding agents (Sheppard and Thomann, 2005) revealed low levels of damage over a large part of the native range of gorse and it was concluded that this guild of insects is unlikely to contain useful biological control agents.

It is now considered unlikely that additional invertebrate species with potential as gorse biocontrol agents will be found in Europe, although one further survey of insects inhabiting gorse pods in NW Spain and Portugal will be conducted in 2007. Seed produced from flowers set in autumn contribute consistently and heavily to the annual seed crop of gorse in New Zealand and elsewhere, and it is surprising that this resource is not exploited by natural enemies in Europe. In contrast, autumn seed production in Europe appears to be inconsistent, and the stochastic nature of the resource may let these seeds escape predation.

As the most recent surveys have shown that the options for additional invertebrate biocontrol agents are limited, surveys for host-specific fungal pathogens of gorse commenced in the European autumn of 2006 (Ireson *et al.*, 2006). Diseased gorse specimens were collected in SW France, NW Spain and northern Portugal to enable isolation, culturing and identification. Surveys for pathogens will also continue during 2007.

## Discussion

Hill *et al.* (2004) pointed out that effective gorse management relies on selecting the most appropriate suite of management tactics for each situation. Where gorse is newly naturalized or of limited distribution, the highest priorities for investment in management should be to determine the extent of the infestation, develop appropriate public policy, contain the distribution, and if possible eradicate the weed. There is little place for biological control here, at least in the short term. Widespread gorse can be managed successfully to protect production or environmental values using conventional methods such as herbicides, although this is expensive and technically difficult. For this reason most gorse infestations worldwide are not managed for economic or environmental gain and yet are already too widespread for containment to be feasible. Biological control appears to be the only means to achieve gorse control in such habitats.

Not all available agents have yet been distributed worldwide (Table 1), but given the lack of immediate success where they have been released, investment in the development of any of these agents should be made

only after critical asessment of their potential contribution to gorse management. With the conclusion of recent surveys, our knowledge of the natural enemies of gorse is assumed to be almost complete. Most of the agents identified to date seem to have lower potential for impact, and appear to be less host-specific than those already released, and there appear to be no compelling new candidate agents. In short, there appears to be no 'classical' biological control solution for gorse in areas where management by conventional means cannot be brought bear as well.

Opportunities remain for augmenting and enhancing classical biological control worldwide through integration with conventional management tactics. Spatially explicit simulation modelling showed that seedling survival (in particular the poor ability of gorse seedlings to compete against grasses) and disturbance were key determinants in the population dynamics of gorse (Rees and Hill, 2001). The model predicted that under a limited range of scenarios of high disturbance and high seedling mortality 75–85% reduction in the annual seed production (initially set at 20,000 seeds $m^{-2}$) as a result of predation by biocontrol agents could lead to a decline of equilibrium cover in the long term.

Davies *et al.* (2005) showed that under laboratory conditions the competitive ability of gorse seedlings growing with grass can be severely reduced by insect attack although this has not been confirmed in the field. The simulation model suggests that by reducing the competitive ability of gorse seedlings in this way, foliage-feeding agents may increase the probability that seed-feeding agents will be effective in achieving long-term control of gorse, bringing the levels of reduction in the annual seed crop required for such control within reach of the known agents.

Gorse population simulations were also sensitive to lifetime fecundity of gorse plants, which is directly related to the maximum age of the plants (Rees and Hill, 2001). None of the control agents released have yet shown a propensity to cause lethal damage to mature plants, but we know little about the chronic effects of these control agents on maximum age in the field. It is possible that sublethal attack may already be reducing the vigour and longevity of gorse, affecting its long-term population dynamics. Additional agents might enhance that effect, even though not greatly damaging in their own right. Management techniques such as the appropriate use of fire, grazing and overseeding may augment this effect (Rees and Hill, 2001; Hill *et al.*, 2004).

High control agent populations that might prove damaging to gorse appear to be constrained from the 'top-down' by predation (in the case of *T. lintearius*), or possibly from the 'bottom-up' by the effect of seasonality and plant quality on the voltinism and intrinsic rate of increase of agents (Hill, 1982; J. Ireson, unpublished data). Even in Hawaii, where predation constraints on *T. lintearius* appear to be absent, severe attack leads to reduction in biomass and flower production, but not plant death. In Australia, the ability of these agents to have any significant long-term impacts on gorse growth and development is considered to be limited without the establishment of additional agents.

The synergy that can exist between conventional control tactics and biocontrol agents in the management of legume shrub weeds such as gorse are clear (Rees and Hill, 2001; Buckley *et al.*, 2004). Integrating weed control techniques may offer the best prospects for long-term control in areas where gorse is actively managed, but the extent to which biological control will play a role in this will only be determined by future research once the full complement of available agents are established.

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# Assigning success in biological weed control: what do we really mean?

**J.H. Hoffmann and V.C. Moran**

*Summary*

The biological control literature is filled with the terms ‘success’ and ‘failure’ used in various guises but in very few cases is the meaning clearly defined. The implicit assumption is that agents generally have failed unless they have caused a substantial decline in the overall abundance of the target weed. This perception is probably a legacy of the early outstanding biological control programmes against cactus weeds, St. John’s wort and, more recently, against floating aquatic weeds. These examples have raised expectations that almost-complete extermination of the target should be the objective of every biological control programme. Consequently, the other types of benefits conferred by biological control are undervalued. One way of ensuring that agents are properly credited is to ask the crucial question: ‘What would the situation have been without any biological control?’ This question provides the focus for assessing the biological control programme against *Opuntia stricta* (Haworth) Haworth in South Africa, using the cactus moth, *Cactoblastis cactorum* Berg., and a cochineal insect, *Dactylopius opuntiae* (Cockerell), which demonstrates how prolonged monitoring can reveal subtle but very real benefits that accrue from otherwise seemingly ineffective agents.

***Keywords***: monitoring, *Opuntia stricta*, *Cactoblastis*, *Dactylopius*.

## Introduction

Almost without exception, early publications on biological control provide definitions that use reduction in either ‘density’ or ‘abundance’ of the target pest as the yardstick of success. Examples include: (i) ‘Utilisation of [natural enemies] for the regulation of host population densities’ (DeBach, 1964); (ii) ‘Effective weed control implies reduction of the population densities of the weed below its level of economic importance’. (Andres and Goeden, 1971); (iii) ‘…the regulation by *natural enemies* of another organism’s population density at a lower level than would otherwise occur’. (DeBach, 1974); (iv) ‘…the science that deals with the role that natural enemies play in the regulation of the numbers of…animal or plant pests’. (Wilson and Huffaker, 1976); (v) ‘Biological control is the use of [natural enemies] to suppress a pest population, making it less abundant…than it would otherwise be’. (Van Driesche and Bellows, 1996).

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The basis of these definitions is a few early projects that resulted in spectacular declines in the density and abundance of the target plants [e.g. the use of *Cactoblastis cactorum* (Berg) against *Opuntia stricta* (Haworth) in Australia; *Chrysolina quadrigemina* (Suffrian) against St John’s wort, *Hypericum perforatum* L., in California] and various projects against aquatic weed species. These ‘overwhelming successes’ established a reputation that biological control is a process that can replace all other control methods. In most up-to-date publications, lectures and discussions, success continues to be equated with substantial reductions in densities of the target weed. A corollary is that projects are perceived as failures when weed densities do not decline, or do so only marginally. Unfortunately, many biological control programmes against weeds fall into this latter category.

More recently, pleas have been made to rationalize what is meant by success (e.g. Hoffmann, 1995; McFadyen, 1998; Briese, 2000; Fowler *et al*., 2000) and to develop ‘more precise performance criteria for the role of biological control in weed management…’ (van Klinken and Raghu, 2006). While this needs to be done if biological control is to receive the recognition it deserves, it seems that ‘old habits die hard’ and that, in the main, biological control practitioners still

hanker after agents that will all but eliminate their target weeds.

Ironically, one of the most 'successful' biological control agents of all time, *C. cactorum*, provides a useful example of how seemingly ineffective agents, in terms of conventional expectations, can provide very real benefits. In this case, the realization was *a posteriori*, but it nevertheless clearly demonstrates how 'success' in biological control need not be an all or nothing process.

*O. stricta* is a widespread weed in South Africa, with one of the most badly affected areas being the Kruger National Park (KNP), the flagship region of South African conservation, where the weed became naturalized during the 1950s. By the 1980s, the problem was out of control in spite of a protracted and expensive herbicide programme that had been in place for several years. In desperation and as a last resort, moves were made to initiate a biological control programme against *O. stricta* in KNP (Hoffmann *et al*., 1998a). This biological control project was potentially straightforward because *O. stricta* had been controlled so superbly by *C. cactorum* in Australia (Dodd, 1940). The moth was already well established on *O. ficus-indica* (L.) Mill. elsewhere in South Africa and was immediately available for introduction into KNP.

The release of *C. cactorum* in KNP during 1987 was greeted with much enthusiasm, fuelled by visible and impressive evidence of larval damage that became apparent over an ever-increasing area of the cactus invasion. The initial euphoria gradually waned as the extent of damage equilibrated at lower levels than were optimistically expected on the basis of the Australian precedents and the cactus remained abundant over a wide area. By 1993, most observers were disillusioned and some were openly critical of the biological control programme, considering it to have aggravated rather than contained, let alone alleviated, the problem. This scepticism and hostility to biological control was the catalyst for the initiation of a research project to quantify the impact of the moth and thereby determine whether or not there have been any benefits from its presence within KNP. The findings of the evaluation programme are presented here.

## Methods

Details of the materials and methods used to accumulate the data presented in the results are given in Hoffmann *et al*. (1998a,b). In essence, the numbers of *C. cactorum* colonies and of plants and fruits of *O. stricta* were monitored over a 9-year period (1993–2001). Measurements of the relative numbers of *C. cactorum* colonies, and of plant size and density were made in two different types of infestations: (1) an area were the cactus had been sprayed with herbicides a year before the initiation of the study (designated the 'treated' area); and (2) an adjacent area where no herbicides had been used (designated the 'untreated' area). The residual population of *O. stricta* in the treated area was sparse and consisted of small plants that had been overlooked during the herbicide treatment. In the untreated area, the plants were large (up to 2.5 m in height) and dense, forming clusters with 100% ground cover over several square meters.

Counts were made along permanent transects which were 100 m in length and 1 m wide. There were five transects in the untreated infestation and ten transects in the treated infestation. The size of the plants was recorded by counting the number of cladodes that made up each plant. To estimate the mean growth rate of undamaged plants, the sizes of individual plants ($n$ = 67) that had not been colonized by *C. cactorum* was measured at the beginning and end of an annual growth cycle.

## Results and discussion

In temperate regions, *C. cactorum* has two generations a year (a short summer one and a long winter one) but in the warm tropical climate of the KNP, the moth passes through two generations in summer and one in winter. Population levels of *C. cactorum* fluctuated both annually and seasonally (Figure 1A). In the treated infestations, the moth's abundance increased between 1993 and 1995 as the populations recovered from being reduced in numbers by the herbicides (Figure 1B), but there was no trend toward an overall increase or decrease in abundance of *C. cactorum* during the study period, 1993–2001.

Despite the presence of *C. cactorum*, there was a steady increase in the density of *O. stricta* plants over the study period in both the untreated and treated infestations, increasing by fourfold and sixfold, respectively (Figure 2). During the same period, there was a substantial increase in the area of the research plots in which *O. stricta* occurred. In the untreated area, quadrat occupancy rose from 17.5% in 1993 to 45.4% in 2001, whereas in the treated area, it rose from 7.9% to 13.7% between 1993 and 2001, showing that the weed was dispersing in spite of damage caused by *C. cactorum*. The biomass of the cactus (measured as cladodes $m^{-2}$) increased in both areas but at a much higher rate in the treated area (Figure 3). Finally, fruit production in the infestations did not diminish as a result of the damage caused by *C. cactorum* (Figure 4). Fruits have been a major concern because one of the immediate objectives in the management of the weed has been to curtail its long-range dispersal by preventing seeding and consequent spread via fruit-eating animals, mainly elephants and baboons.

When considered in the light of conventional definitions of success in biological control (i.e. reduction in density or abundance of the target weed), *C. cactorum* on *O. stricta* in KNP must rank as an outright failure. So how can the situation be perceived differently? One

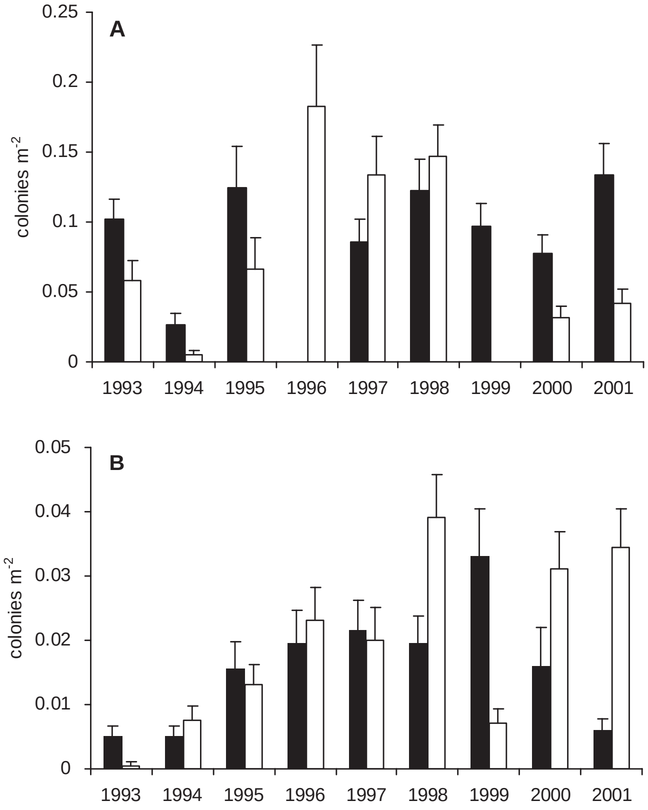


**Figure 1.** Abundance of *Cactoblastis cactorum* (mean ± 1 SE colonies $m^{-2}$) in an untreated infestation (A) and a herbicide-treated infestation (B) of *Opuntia stricta* in KNP between 1993 and 2001 (solid bars = winter; open bars = summer).

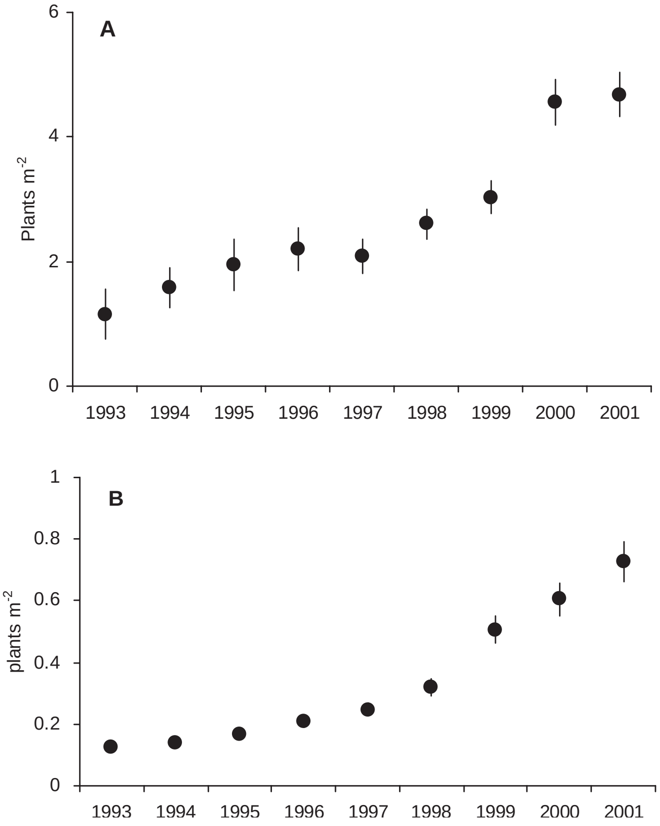


**Figure 2.** The density of *Opuntia stricta* plants (mean ± 1 SE plants $m^{-2}$) in an untreated infestation (A) and a herbicide-treated infestation (B) in KNP between 1993 and 2001.

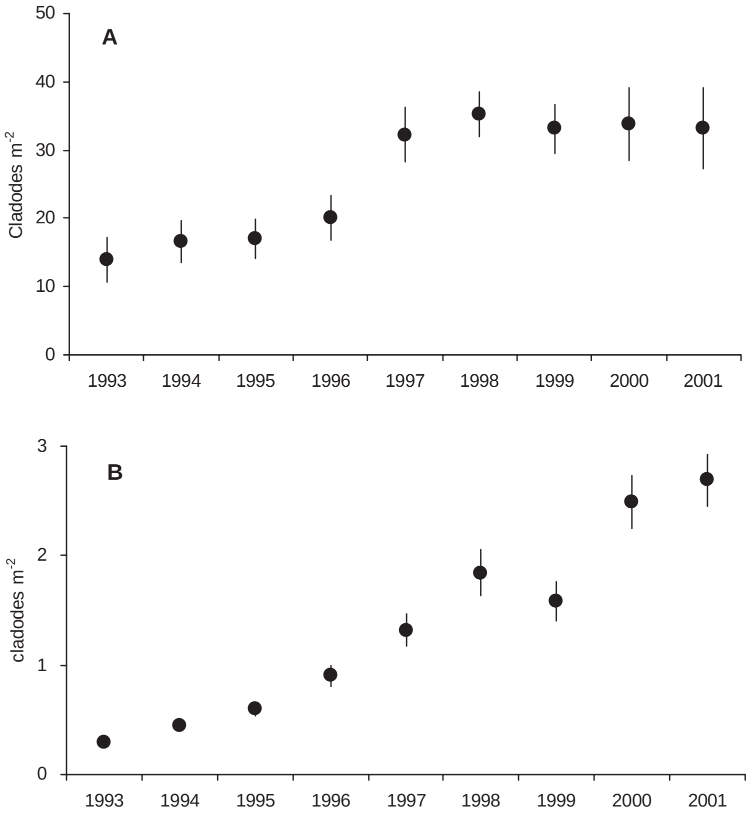


**Figure 3.** The biomass (mean ± 1 SE cladodes $m^{-2}$) of *Opuntia stricta* in an untreated infestation (A) and a treated infestation (B) in KNP between 1993 and 2001.

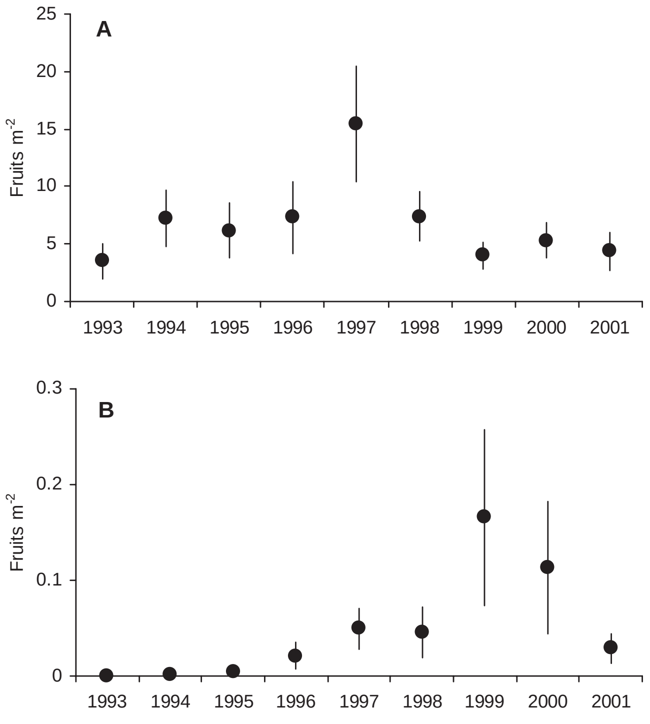


**Figure 4.** Fruit production (mean ± 1 SE numbers $m^{-2}$) on *Opuntia stricta* in an untreated infestation (A) and a herbicide-treated infestation (B) in KNP between 1993 and 2001.

way is to ask 'What would the situation have been without any biological control?' and to use a simple spreadsheet model to estimate how much cactus there would have been if *C. cactorum* was not in the system.

The counts of cladodes on healthy, undamaged *O. stricta* plants in KNP showed that there is an annual increment of 1.8. This value can be used to calculate the expected increase in biomass of the cactus over a given time assuming absence of *C. cactorum*. Comparisons of expected and observed biomass over an 8-year period show that there is an escalating difference with time (Figure 5), which is attributable to damage caused by *C. cactorum*. A substantial divergence accrues even when annual growth of the cactus is reduced to a factor of 1.6 each year (i.e. 90% of capacity). The comparisons show that the infestations of the weed were much less prolific than they would have been without biological control and, even though the problem was getting worse, the rate at which this was happening had been substantially curtailed by *C. cactorum*.

The reduction in growth rates due to *C. cactorum* took on special significance when biological control was integrated with herbicide treatment of the weed. The details of such an integrated programme are described by Hoffmann *et al*. (1998b) and Lotter and Hoffmann (1998). Essentially the process entailed less frequent applications of herbicides and treatment of only the largest fruit-bearing plants while leaving small plants, which are difficult to locate, for *C. cactorum*. This procedure resulted in a substantial financial saving and enabled coverage of a greater area of the infested parts of the park with the same allocation of resources.

This study has shown that qualitative assessments that regarded *C. cactorum* as something of a failure were not justified. The key element in changing this misconception was to ask 'What would the situation have been without biological control?' rather than 'What has been achieved?' This approach forces the observer to consider scenarios of where the weed would be without biological control and therefore to look at the system from a different and more-telling perspective. In the case of *C. cactorum*, it has been possible to show, through careful evaluation over almost a decade that the moth was having a dramatic effect, even though the weed remained abundant in the presence of this agent acting alone.

More recently, the situation has changed dramatically with the introduction of a cochineal insect, *Dactylopius*

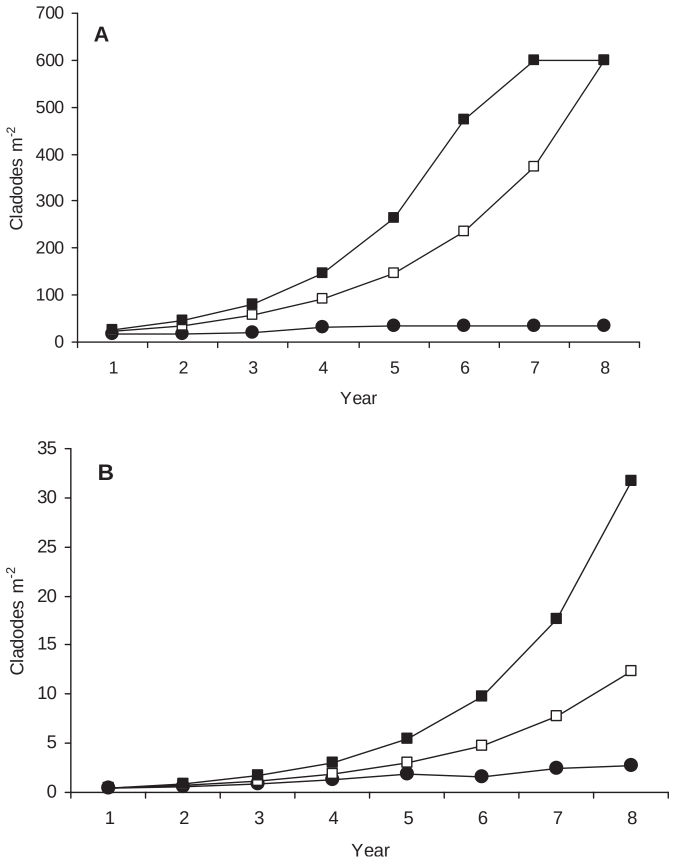


**Figure 5.** Observed (closed circles) and expected (closed squares = growth at 100% of capacity; open squares = growth at 75% of capacity) densities of cladodes (number $m^{-2}$) in an untreated infestation (A) and a herbicide-treated infestation (B) of *Opuntia stricta* in KNP over an 8-year period.

*opuntiae* (Cockerell), into KNP. This step has resulted in a massive decline in the abundance of *O. stricta* in KNP (unpublished results) and the weed is now considered by everyone to be under excellent biological control. The situation continues to be monitored to confirm that this level of control is sustainable. The integrated control programme has been replaced by a fully fledged biological control initiative with cochineal being manually dispersed at every opportunity and herbicides no longer used. *C. cactorum* persists in the system and the interactions of the two agents are being monitored.

In spite of this recent drastic change in the status of *O. stricta* in KNP, the message remains that we need to get away from the perception that a reduction in density is the primary, if not the sole, requirement for success in biological control. In doing so, the status of many of our supposedly unsuccessful agents is going to change and biological control is going to be perceived much more favourably, both by ourselves, by the public at large and, perhaps most importantly, by the people holding the 'purse strings'.

## Acknowledgements

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# Combination of a mycoherbicide with selected chemical herbicides for control of *Euphorbia heterophylla*

**K.L. Nechet,[1] B.S. Vieira,[1] R.W. Barreto,[1] E.S.G. Mizubuti[1] and A.A. Silva[2]**

*Summary*

The leaf-spot fungus *Lewia chlamidosporiformans* is being developed as a mycoherbicide for wild poinsettia (*Euphorbia heterophylla*), a serious weed of many crops, and particularly of soybeans, in Brazil. A comparative study of the levels of control of wild poinsettia obtained for the fungus alone, the fungus plus selected herbicides and these herbicides alone was undertaken. The levels of control obtained with application of the fungus alone was equivalent to the chemical herbicides fomesafen, carfentrazone and atrazine for plants with up to five leaves, and equivalent to the herbicide glyphosate for plants with five to ten leaves for one of the weed populations being tested. The fungus alone had a better performance than both imazethaphyr and fomesafen. The combination of fomesafen with the fungus produced complete control for the three weed populations, whereas its combination with imazethaphyr significantly improved the control levels as compared with those obtained with imazethaphyr alone. Another experiment performed in microplots in the field, explored the combination of *L. chlamidosporiformans* with fomesafen and clorimuron-ethyl for the control of an imazethaphyr-resistant biotype of the weed. The best control levels were achieved with the application of an equivalent of 300 l/ha of a suspension of conidia ($2.5 \times 10^5$ conidia/ml) in a solution of fomesafen (25% of recommended dose). Death of all plants resulted from such application after 10 days.

***Keywords***: *Lewia*, fungus, weed management, wild poinsettia, bioherbicide.

## Introduction

*Euphorbia heterophylla* L. (wild poinsettia, local name in Brazil amendoim-bravo or leiteiro) is a plant native to the Neotropics that became an aggressive invader of important crops such as corn, sugarcane, common bean and soybean in Brazil. It was recognized as a potential biocontrol target with the pioneering development of a mycoherbicide aimed at controlling wild poinsettia in Brazil (Yorinori, 1985, 1987; Yorinori and Gazziero, 1989). This was based on *Bipolaris euphorbiae* (Hansford) Muchovej. Although interest in this fungus still remains (Marchiori *et al.*, 2001; Nechet *et al.*, 2006), this research never resulted in a commercially viable product. Chemical control through application of acetolactate synthase (ALS)-inhibiting herbicide remains the method of choice for control of *E. heterophylla* in soybeans. Herbicides with this action mechanism are efficient in low doses, have a low toxicity for mammals and are selective for several important crops (Saari *et al.*, 1994). Unfortunately, the continuous use of this group of herbicides led to the widespread emergence of wild poinsettia populations that are resistant to these products, rendering them ineffective in many situations (Gazziero *et al.*, 1997; Melhorança and Pereira, 1999).

Problems with herbicide resistance represent windows of opportunity for the development and use of mycoherbicides, either separately or in mixture with chemical herbicides (Charudattan, 2001). Surveys of the mycobiota of *E. heterophylla* in Brazil yielded additional fungi that might be of interest for use as mycoherbicides (Barreto and Evans, 1998). An isolate

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of *Lewia chlamidosporiformans* Vieira and Barreto, a fungus recently described (Vieira and Barreto, 2005), was selected as a potential mycoherbicide. It was capable of causing high mortality in nine populations of *E. heterophylla*, including one resistant to ALS-inhibiting herbicides.

Combining a biocontrol agent with a chemical herbicide may result in the reduction of the dose of the chemical herbicide needed to control a specific weed. It may also serve to increase the spectrum of weeds controlled by the chemical product or increase its efficiency of control of a problem weed species (Hoagland, 1996). It was hypothesized that in the case of *E. heterophylla*, *L. chlamidosporiformans* might have such an effect when combined with ALS-inhibiting herbicides, as well as other groups of herbicides.

## Materials and methods

### Isolate of *L. chlamidosporiformans* and biotypes of *E. heterophylla*

An isolate of *L. chlamidosporiformans* (KLN06) was previously selected, among six other isolates, because it was capable of causing high disease severity when tested against nine different populations of *E. heterophylla* (including one known to be resistant to ALS-inhibiting herbicides and one resistant to *B. euphorbiae*). KLN06 was the isolate used in the experiments described here.

Three different populations of *E. heterophylla* were selected to be used in the first experiment: EKLN19, previously selected as susceptible to *L. chlamidosporiformans*, *B. euphorbiae* (Hansford) Muchovej and *Sphaceloma poinsettiae* Jenkins and Ruehle; ETRB – resistant to *B. euphorbiae*, collected in experimental fields of Paraná; and ERH – resistant to ALS-inhibiting herbicides. In the second experiment, only EKLN19 was used. Seeds to be used in the experiments were harvested from greenhouse-grown potted plants and kept at 5°C until use. Plants used in the experiments were produced from pregerminated seeds that were then transferred to 500 ml pots containing sterile soil and the plants were maintained in a greenhouse (26 ± 2ºC) and watered daily in the first experiment.

### Inoculum production

Spores of *L. chlamidosporiformans* to be used as inoculum in the experiments were produced with a biphasic technique modified from Walker (1980) as follows: ten culture disks obtained from the margin of 7-day-old cultures grown in VBA (Pereira *et al.*, 2003) were transferred to each of a series of Erlenmeyer flasks containing 100 ml of VB (the same medium described by Pereira *et al.*, 2003, but without agar). The Erlenmeyers were left on a shaker at 140 rpm for 7 days at room temperature. After this period, the mycelial mass was blended together with the remaining liquid medium in each flask and poured and spread onto 20 × 28 cm aluminium trays, each already containing 100 ml of solidified VBA. Trays were kept in a controlled temperature room at 26 ± 2ºC under a 12-h photoperiod (light from two 40-W daylight fluorescent lamps and two 40-W fluorescent, near ultraviolet light lamps). After 2 days, spores were collected by pouring 50 ml of sterile water on the culture surface and scraping it with a rubber spatula. The resulting suspension was then filtered through two layers of cheesecloth and the final concentration of the suspension was evaluated and adjusted for the experiments.

### Wild poinsettia control with mycoherbicide, selected herbicides and their combination – greenhouse

Only herbicides that did not inhibit spore germination in a previous experiment were used in this experiment. These were atrazine, carfentrazone, fomesafen, glyphosate and imazethaphyr. Plants belonging to the three populations above were sprayed with (1) the herbicides alone; (2) the fungus alone at a concentration of $2 \times 10^5$ conidia/ml supplemented with Assist – 3% (mineral oil) and Break thru® – 320 µl/100 ml (an organosilicone surfactant); and (3) with mixtures of the fungus inoculum on the concentration mentioned above with each of the herbicides diluted in water supplemented with Assist – 3% and Break thru® – 320 µl/100 ml. Plants were sprayed at the end of the afternoon, left outside to be exposed to natural dew formation and moved next morning to a greenhouse (26 ± 2ºC). The experiment was carried out in a completely randomized design with a factorial of 12 treatments, three plant populations and five replications per treatment. Each replication consisted of one pot containing one plant. Plants were treated at an age of 4 weeks (four leaf stage).

The evaluations were made 5 and 10 days after the application of the treatments using a scale from 0 to 10 (Table 1). The nonparametric Kruskal–Wallis (KW) test was done on the rating values for each isolate and time after inoculation combination. When KW tests were significant, multiple comparisons of the means were conducted to assess differences among treatments at $\alpha = 0.05$. All nonparametric analyses were conducted using the R package® ver 2.3.1 (R Development Core Team, 2006).

### Wild poinsettia control with mycoherbicide, selected herbicides and their combination – field

The experimental unit consisted of an area of 1.5 $m^2$ (1.5 × 1.0 m), where three parallel 0.5-cm-deep rows set

**Table 1.** Scale of notes for evaluation of the control of populations of *Euphorbia heterophylla*.

| Rank | Description of ranking | Control percentage |
|---|---|---|
| 0 | No symptom | 0 |
| 1 | Yellowing | 10 |
| 2 | Terminal buds not damaged, less than 20% of the leaves showing injuries | 20 |
| 3 | Terminal buds not damaged, 20–50% of the leaves showing injuries | 30 |
| 4 | Terminal buds not damaged, more than 50% of the leaves showing injuries | 40 |
| 5 | Terminal buds not damaged, all leaves with injuries | 50 |
| 6 | Terminal buds necrosed, leaves healthy | 60 |
| 7 | Terminal buds necrosed, less than two leaves with injury | 70 |
| 8 | Terminal buds necrosed, more than two leaves injured | 80 |
| 9 | Terminal buds necrosed, stems green, complete defoliation | 90 |
| 10 | Dead plants | 100 |

0.5 m apart were prepared for planting. An amount of fertilizer equivalent to 20 kg/ha of N, 100 kg/ha of $P_2O_5$ and 100 kg/ha of $K_2O$ was added and a row equivalent to 25–30 soybean seeds/m was sown. Additional parallel rows, 25 cm to each side of the soybean rows, were prepared where ETRB *E. heterophylla* seeds (equivalent to 30 seeds/m) were planted. Treatments consisted of spraying with the following:

1. suspension of $2.5 \times 10^5$ conidia/ml of *L. chlamidosporiformans*;
2. herbicide clorimuron-ethyl (Classic – 80 g/ha);
3. herbicide clorimuron-ethyl (Classic – 80 g/ha) + $2.5 \times 10^5$ conidia/ml of *L. chlamidosporiformans* suspended in the herbicide solution;
4. herbicide fomesafen (Flex, 1.0 l/ha – recommended dose);
5. herbicide fomesafen (Flex, ¼ of recommended dose);
6. herbicide fomesafen (Flex, ¼ of recommended dose) + $2.5 \times 10^5$ conidia/ml of *L. chlamidosporiformans* suspended in the herbicide solution;
7. herbicide fomesafen (Flex, ¼ of recommended dose) + $2.5 \times 10^6$ conidia/ml of *L. chlamidosporiformans* suspended in the herbicide solution;
8. control 1: soybean challenged with wild poinsettia sprayed with water;
9. control 2: soybean growing free of wild poinsettia competition sprayed with water.

The volume of solution/suspension sprayed on each treatment was equivalent to an application of 300 l/ha. Water used to suspend or dilute the active ingredient or inoculum was always supplemented with Assist – 3% (mineral oil) and Break thru® – 320 µl/100 ml. Plants were inoculated at the age of 2 weeks (three to four leaf stage) at the end of the afternoon. Evaluations were made 5 and 10 days after the application of the treatments using the criteria adopted by SBCPD (legend, Table 2). The experiment was carried out in a completely randomized design with five repetitions; each repetition was as described above. Data obtained were graphed with the aid of Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA).

**Table 2.** Percentage of control of *Euphorbia heterophylla* in the field after spraying with *Lewia chlamidosporiformans*, selected herbicides and fungus–herbicide combinations.

| Treatment | Control percentage | | | |
|---|---|---|---|---|
| | Five days | | Ten days | |
| | Percentage control | Control description | Percentage control | Control description |
| Clorimuron-ethyl | 80 | B | 70 | C |
| Clorimuron-ethyl + fungus ($10^5$ conidia/ml) | 98 | A | 99 | A |
| Fomesafen standard dose | 100 | A | 100 | A |
| Fomesafen ¼ dose | 94 | A | 86 | B |
| Fomesafen ¼ dose + fungus ($10^5$ conidia/ml) | 98 | A | 100 | A |
| Fomesafen ¼ dose + fungus ($10^6$ conidia/ml) | 92 | A | 98 | A |
| Fungus ($10^5$ conidia/ml) | 97 | A | 99 | A |
| Control | 0 | E | 0 | E |

Capital letters represent the control description according to SBCPD (1995). A = Excellent control or total control of the target species (86–100%); B = Good control of the target-species, acceptable for an infested area (66–85%); C = Moderate control of the target-species, insufficient for the infested area (41–65%); D = Control of the target-species deficient or irrelevant (0–40%); E = No control (0%).

# Results

## Wild poinsettia control with mycoherbicide, selected herbicides and their combination – greenhouse

There were differences among treatments at both 5 and 10 days. The *P* values were less than 0.0001 for all tests and the chi-square values for populations TRB, KLN19 and RH at 5 days were 58.9, 59.0 and 58.8, respectively. At 10 days, the chi-square values were 58.8, 55.8 and 56.3, respectively.

**Five days:** When assessing plants of the TRB population, the control differed from the treatments: Atrazine + *L. chlamidosporiformans*, Carf + *L. chlamidosporiformans*, Flex + *L. chlamidosporiformans* and Pivot + *L. chlamidosporiformans*. All other pairwise comparisons involving the control were not significant. The only mixture of herbicide plus *L. chlamidosporiformans* that did not differ from the control was Glyphosate + *L. chlamidosporiformans*. The pairwise comparisons involving *L. chlamidosporiformans* vs Atrazine + *L. chlamidosporiformans* and Atrazine vs *L. chlamidosporiformans* + Imazethaphyr were significant.

When assessing plants of the KLN19 population, the control differed from the treatments: *L. chlamidosporiformans*; Atrazine; Atrazine + *L. chlamidosporiformans*; Carfentrazone; Carfentrazone + *L. chlamidosporiformans*; Fomesafen + *L. chlamidosporiformans*; and Glyphosate + *L. chlamidosporiformans*. All other pairwise comparisons involving the control were not significant. Fomesafen, Glyphosate, Imazethaphyr and Imazethaphyr + *L. chlamidosporiformans* treatments did not differ from the control. The pairwise comparisons involving Atrazine vs Atrazine + *L. chlamidosporiformans* and Atrazine + *L. chlamidosporiformans* vs Imazethaphyr were significantly different.

When assessing plants of the RH population, the control differed from the treatments: Atrazine + *L. chlamidosporiformans*; Carfentrazone + *L. chlamidosporiformans*; Fomesafen + *L. chlamidosporiformans*; and Imazethaphyr + *L. chlamidosporiformans*. All other pairwise comparisons involving the control were not significant. Similarly to the reported for KNL19, pairwise comparisons involving Atrazine vs Atrazine + *L. chlamidosporiformans* and Atrazine + *L. chlamidosporiformans* vs Imazethaphyr were significantly different.

**Ten days:** For both the TRB and RH population, the control differed from the following treatments: Atrazine + *L. chlamidosporiformans*; Carfentrazone + *L. chlamidosporiformans*; Fomesafen + *L. chlamidosporiformans*; and Imazethaphyr + *L. chlamidosporiformans*. All other pairwise comparisons involving the control were not significant. Pairwise comparisons between Atrazine vs Atrazine + *L. chlamidosporiformans*; Atrazine + *L. chlamidosporiformans* vs Imazethaphyr; and Atrazine vs Imazethaphyr were significantly different. For the KLN19 population, there were no pairwise differences between treatments.

All plants of population EKLN19 died 5 days after being sprayed with *L. chlamidosporiformans* as well as with the herbicides atrazine, carfentrazone and with the mixtures of these herbicides with the fungus. Equivalent levels of control were observed for the populations ERH and ETRB submitted to the same herbicides and with their mixture with the fungus. The level of control obtained with the pure application of *L. chlamidosporiformans* in those populations was lower then that obtained for EKLN19. Control was inadequate for the application of fomesafen and imazethaphyr, confirming their known inefficiency for the control of *E. heterophylla* plants at the stage of development of application in this experiment (three to four leaf stage). Nevertheless, complete control of wild poinsettia was obtained when fomesafen was mixed with *L. chlamidosporiformans* (Figure 1). The combination of imazethaphyr with the fungus led to significant increase in control levels for the three weed populations, whereas they were totally inadequate for imazethaphyr alone. Ten days after the inoculation, the level of control, obtained for the combination of *L. chlamidosporiformans* with imazethaphyr, was considered acceptable for EKLN19 and for ERH although still insufficient for ETRB.

Glyphosate sprays led to total control of the three wild poinsettia populations, after 10 days when applied alone, but control was inadequate for EKLN19 and ERH initially, that is 5 days after the application. However, when mixed with the fungus, glyphosate gave complete control of all weed populations in just 5 days, speeding weed control.

## Wild poinsettia control with mycoherbicide, selected herbicides and their combination – field

All *E. heterophylla* plants treated with the fungus + fomesafen (¼ of the dose) were killed 10 days after being sprayed (Table 2). In the first evaluation, 5 days after the application, levels of control obtained for the mixtures fungus + fomesafen (¼ of the dose) and fomesafen alone (¼ of the dose) did not differ statistically. Nevertheless, on the second evaluation, 10 days after the applications, total control was reached only with the mixtures fungus–herbicide. Level of control obtained with fomesafen alone (¼ of the dose) and clorimuron-ethyl alone decreased from the first evaluation to the second evaluation indicating a recovery of the weed population from the damage caused by the herbicides. Meanwhile, a tendency of increased control was noted for treatments involving the use of *L. chlamidosporiformans* probably mirroring the progressive advance of the disease. The level of control obtained with the isolated application of *L. chlamidosporiformans* was also ranked as excellent and statistically equivalent

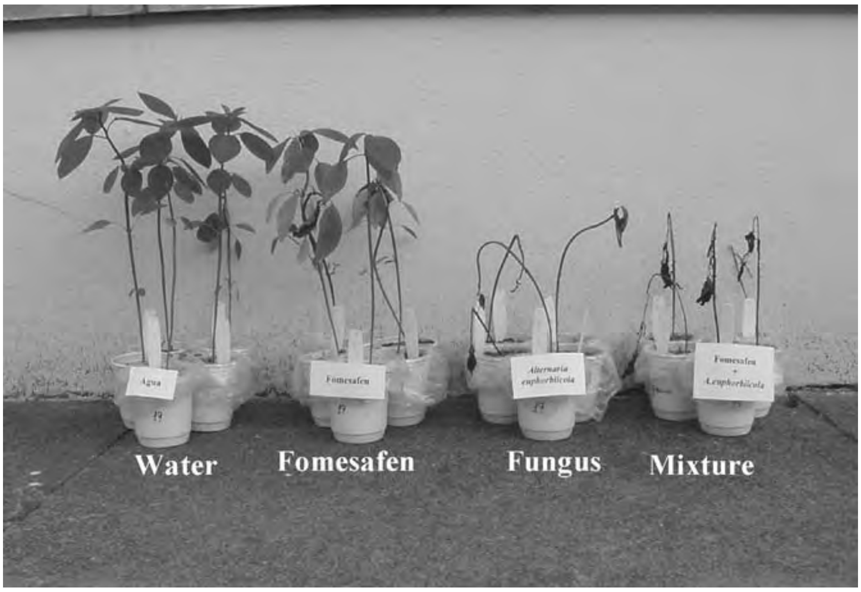


**Figure 1.** Control of population EKLN19 of *Euphorbia heterophylla*, 5 days after spraying with water, fomesafen, a conidial suspension of *Lewia chlamidosporiformans* and a mixture of fomesafen + *L. chlamidosporiformans*.

to that obtained with the application of the herbicide fomesafen at the recommended dose and superior to that obtained with the application of ¼ of the dose of this herbicide (Table 2). The combination of the herbicide clorimuron-ethyl with the fungus resulted in a percentage of control higher than that obtained for the herbicide alone.

## Discussion

The level of control obtained with application of *L. chlamidosporiformans* varied for each weed population, but when in combination with various herbicides included in the test, the level of control was either kept as complete control or increased as compared with the herbicide alone. The efficiency of control of *E. heterophylla* by fomesafen is seriously restricted if applications occur at a later phenological state of the plant, as observed in greenhouse experiment (where older plants were used) and Table 2 (where younger plants were used), but total control was achieved with a mixture of fomesafen and *L. chlamidosporiformans* of older plants (Figure 1). The fungus in mixture with glyphosate also allowed the anticipation of the wild poinsettia's control by this product. These results are highly encouraging for an integration of biological and chemical control of wild poinsettia. This work seemingly is the first to focus on that aspect in the development of a mycoherbicide in Brazil.

The herbicide imazethaphyr did not control the population ERH (known to be resistant to this product) and the population ETRB. The population ETRB was selected because it was known to be resistant to *B. euphorbiae*; the present results show that it is also resistant to imazethaphyr. The level of control of wild poinsettia was significantly increased when *L. chlamidosporiformans* was mixed with that herbicide, but control percentage was for most situations lower than that obtained by the fungus alone. The incompatibility between mycoherbicide and chemical herbicides is still a little investigated subject, but it is known that herbicides can interfere with disease development, either because of a direct toxicity to the pathogen or indirectly by triggering defense responses in the plants (Sanogo *et al.*, 2000). The negative effect of imazethaphyr observed on *L. chlamidosporiformans* was indirect because this herbicide did not inhibit conidial germination in vitro (unpublished results) in the recommended dose. Possibly, some plant defense mechanism of the plant is activated after the application of imazethaphyr and this slowed disease development. Conversely, it was recently observed that imazethaphyr applications can increase significantly the severity of attack to soybean by *Rhizoctonia solani* Khun both in resistant and susceptible cultivars (Bradley *et al.*, 2002).

*Lewia chlamidosporiformans* can become an important tool in the management of populations of *E. heterophylla* resistant to (ALS)-inhibiting herbicides in separate applications as a mycoherbicide, but further investigation of its interaction with imazethaphyr as a possible combination is needed. Other options deserving to be investigated are the use of smaller doses of imazethaphyr with the fungus and the effect of separate sequential application of fungus and herbicide or herbicide followed by the fungus.

The second experiment also yielded encouraging results both for the potential of the mycoherbicide alone and for fungus–herbicide combinations. Often the best alternative for weed management depends on the association of different methods of control (Silva and Altoé, 1993). In this experiment, one of the most noteworthy results was achieved with the combination of *L. chlamidosporiformans* and fomesafen at ¼ of the recommended dose. Complete control of wild poinsettia was achieved even with a significant reduction of the use of the chemical herbicide and a relatively small inoculum concentration for the fungus. Except for the isolated application of clorimuron-ethyl that did not result in an appropriate control of the weed, the other treatments resulted in a good control of the weed. Adding a relatively low concentration of fungus inoculum to clorimuron-ethyl also led to complete control of wild poinsettia.

Although a series of studies involving *L. chlamidosporiformans* have been yielding excellent results that are presently being published (see Vieira *et al.*, this volume) and firmly indicate that this fungus may be used soon as a mycoherbicide, particularly against herbicide-resistant populations of *E. heterophylla*, further work is still necessary. Improvement in mass production, formulation and application technology are still required as well as further studies on fungus–herbicide combinations.

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# Sustainable management based on biological control and ecological restoration of an alien invasive weed, *Ageratina adenophora* (Asteraceae) in China

**F. Zhang,[1] W.-X. Liu,[2] F.-H. Wan[2] and C.A. Ellison[3]**

*Summary*

Crofton weed, *Ageratina adenophora* (Sprengel) R. King and H. Robinson, originally from Central America, was introduced into China in the 1940s. The weed spreads rapidly and is seriously damaging grasslands and hindering livestock production in southwestern China. To tackle the weed problem and allow the sustainable use of pastures, an integrated strategy, based on biological control and habitat restoration, is being explored. In 1983, a gall fly *Procecidochares utilis* Stone, originating from Mexico, was introduced from Tibet into Yunnan Province for the control of crofton weed. The current efficacy of this agent was investigated, but no significant control effect was found due to more than 60% parasitism by native parasitoids. Surveys were undertaken to identify any indigenous fungal pathogens infecting the weed. At least six strains of *Alternaria alternata* (Fr.) Keissler and a *Pestalotiopsis* sp. were isolated from leaves. One strain of *Alt. alternata* was selected for further study as a prospective mycoherbicide. Field trials on ecological restoration using competitive native plants and forages showed that *A. adenophora* was less interspecifically competitive than *Setaria sphacelata* (Schum.) Stapf. ex Massey cv. Narok.

***Keywords***: crofton weed, natural enemies, competitive weed replacement, IPM.

## Introduction

The crofton weed *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Asteraceae) (Synonym *Eupatorium adenophorum*) from Central America was introduced into China in the 1940s. The weed has distributed rapidly and is seriously damaging grasslands and livestock production in the southwestern China provinces of Yunnan, Guizhou, Sichuan, Guangxi and Tibet (Xiang, 1991; Qiang, 1998). Its successful invasion can be attributed to its strong adaptability, competitive ability in new invaded areas, abundant seed production and paucity of natural enemies, compared with its native range (Qiang, 1998; Wang, 2005). *A. adenophora* is still continuing to spread with an average expansion rate of 20 km/year throughout the south and middle subtropical zones, and 6.8 km/year in north subtropical areas (Wang and Wang, 2006). It has invaded meadow, forest and wetland, forming single dominant communities over a short period of time and thus has caused the decline and disappearance of the original plant community. It releases allelopathic substances into the soil, via the roots, which can inhibit seed germination of neighbouring plant species (Tripathi *et al.*, 1981; Baruah *et al.*, 1994; Zheng and Feng, 2005). However, allelopathic effects on susceptible plants, such as *Chromolaena odorata* (L.) R.M. King and H. Robinson, *Bidens pilosa* L., *Ageratum conyzoides* L. and *Gynura* sp., were not significant at the seedling stage of *A. adenophora* in shaded plots (Wang and Feng, 2006). This weed also exhausts arable soil fertility due to its strong absorption of soil nutrients (Liu *et al.*, 1989). Moreover, *A. adenophora* threatens the health of livestock as the branches and leaves are poisonous to domestic animals, particularly horses (O'Sullivan, 1979; Kaushal *et al.*, 2001; Wang, 2005).

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*A. adenophora* has become one of the worst invasive alien species (IAS) in China. In 2003, *A. adenophora* was one of 15 IAS listed in the 'White Paper of Primary IAS', released by State Environmental Protection Administration of China. Enormous efforts have been made by Central Government (through the Ministry of Agriculture) and local government, to control and/or eliminate *A. adenophora* in newly invaded areas, and control methods have been relying on chemical herbicides and mechanical removal. However, *A. adenophora* continues to spread, expanding its range principally toward the vast area of southern and southcentral China (Wang and Wang, 2006). This paper summarizes a sustainable management approach, based on biological control and ecological restoration, currently being developed for crofton weed in China.

## Sustainable management measures for *Ageratina adenophora*

### Classical biological control with a gall fly, *Procecidochares utilis* Stone

*Procecidochares utilis*, originating from Mexico, is a gall fly that forms galls in the stem of *A. adenophora*. It lays eggs on the stem apex and on hatching the larvae tunnel into the stem. In response to larvae presence, a gall forms in the stem, which may contain from 1 to 23 larvae (Bennett and Van Staden, 1986). Galls have been shown to cause severe stunting, a reduction in flowering and seed set and may result in ultimate death of the plant when they occurred in large numbers (Bennett and Van Staden, 1986). In 1984, *P. utilis* was introduced from Nienamu county Tibet into Kunming, Yunnan Province, for the control of crofton weed (Wei *et al.*, 1989). Since its introduction, field populations of *P. utilis* have been successfully established in the introduced areas in Yunnan, Guizhou and Sichuan provinces (Dai *et al.*, 1991; Chen and Guan, 1994; Li *et al.*, 2006; Wang *et al.*, 2006). The gall fly is multivoltine with the number of generations varying across the weed's invaded range in China (four in Kunming, Yunnan Province; five in southwest Guizhou Province; and six generations in the warmer lowlands of Sichuan).

A field study in 1990–1994 showed that infestation rate of *P. utilis* in the released area was approximately 10–37% on average, but could reach up to 85–96%. The average annual dispersal rate of *P. utilis* was 20–25 km (max. 40 km) from original release sites, facilitated by the southwest monsoon (Chen and Guan, 1994). Infected plants had a significantly decreased flower number, as well as seed weight, size and percentage germination (Wang *et al.*, 2006). It was concluded that the gall fly plays an important role in suppressing population growth of crofton weed as well as reducing its rate of spread (Chen and Guan, 1994; Wang *et al.*, 2006).

However, the dispersal of the gall fly has been lagging behind the spread of *A. adenophora* (Li *et al.*, 2006). In addition, the overall impact on the weed in the field has been disappointing, and a number of contributory factors have been investigated.

- *P. utilis* shows highly selective oviposition behaviour, leading to a restricted population size in the field. The gall fly will only oviposit on the stem apex, showing a preference for younger plant branches. This results in a reduction in eggs laid during the flowering season of the plant, when only less optimal oviposition sites are available (Wei *et al.*, 1989; Li *et al.*, 2006).
- Li *et al.* (2006) argued that *P. utilis* had no significant effects on the number of blossom branches, capitula and seeds produced by *A. adenophora*, which indicated limited impact on reproduction and spread of the weed.
- Significant differences were found by Li *et al.* (2006) in the infestation rates based on individual plants (71.7%) and plant branches (17.3%). Since *A. adenophora* produces a mean of 21 branches per plant, this suggests that *P. utilis* is unlikely to have a significant impact on the whole weed population.
- Recent field surveys have also revealed that *P. utilis* is parasitized by indigenous parasitoids; *Torymoides kiesenzuetteri* (Mayr), *Eupelmus urozonus* Dalman, *Bracon* sp., *Eurytoma* spp., *Sphegigaster* spp. and *Pteromalus* spp., which might also explain its lower-than-anticipated control effect. B.P. Li (2006, personal communication) observed heavy parasitism of more than 60% in the field.

### Biological control with fungal pathogens

Fungal pathogens of *A. adenophora* have been studied since the first report of *Phaeoramularia eupatorii-odorati* (Yen) Liu and Guo (= *Cercospora eupatorii* Peck) in Australia in 1954 (Auld, 1969). This fungus was accidentally introduced, probably carried by *P. utilis* adult flies when they were introduced from Hawaii. This leaf-spot fungus is widespread and is responsible for significant premature leaf abscission, and is particularly prevalent in cleared areas. It is believed that the impact of the weed in Australia is considerably lessened by the pathogen (R.E. McFadyen, 2007, personal communication). It was released in South Africa in 1987, but although it has established, its impact on weed populations is minimal (Julien and Griffiths, 1998).

In Yunnan province, *P. eupatorii-odorati* is a common disease of *A. adenophora* and reaches epidemic levels during June and July. It causes reductions in the photosynthetic rate, transpiration rate and chlorophyll content, thus reducing plant height and number of leaves and flowers (Yang and Guo, 1991; Guo *et al.*, 1992). In laboratory studies, the optimal temperature for

conidia germination of *P. eupatorii-odorati* was 25°C and the optimal pH was 5.0, and, like most pathogens, it requires free water on the plant surface to develop infection (Guo *et al.*, 1992). This study recommended that the ideal time, considering temperature and water requirements, to release this pathogen into the area where it does not occur in Yunnan province, was July and August.

In China, indigenous fungal pathogens infecting *A. adenophora*, which may have potential as mycoherbicides to control the weed (Qiang, 1998), have also been investigated. At least six strains of *Alternaria alternata* (Fr.) Keissler and a *Pestalotiopsis* sp. were isolated from leaves of *A. adenophora* (Wan *et al.*, 2001; W.X. Liu, unpublished data). Necrotic leaf spots on *A. adenophora* leaves were observed 24 hours after treatment with a toxin extract produced by *Alt. alternata*, at a dosage of 50–300 μg/ml (Dai *et al.*, 2004). Recent work by Qiang *et al.* (2006) has found that mycelial preparations of *Alt. alternata* are more effective infection propagules than conidia. The assessment of the potential of these local fungal pathogens is at the early stages of laboratory research and small-scale field trials.

## Ecological restoration by competitive replacement with native plants and forage grasses

Ecological restoration involves the re-establishment of the structure and function of an ecosystem, primarily to achieve a pre-disturbance state. In China, studies have focused on restoration of both natural habitats with the native flora, and pastureland with forage grasses. The selection of alternative plants has been based on a number of factors:

- *A. adenophora* has a strong allelopathic effect on other plants, and degrades the soil; therefore, replacement plants need to be tolerant of the prevailing soil conditions, as well as being strongly competitive.
- Seeds of crofton weed are very sensitive to sunlight during germination and demonstrate less interspecific competitive ability at the seedling stage. Therefore, the chosen alternative plants should be easy to grow, with a high rate of growth, and a canopy density that can reach 70% within a short period.
- In the pasturelands of southwestern China, it is also critical to consider field application and technology dissemination within the rural communities. Therefore, to enhance development in rural areas, it would be favourable to choose alternative plants that have a high economic value.

Following these criteria, the candidate species selected were *Brachiaria subquadripara* (Trin.) Hitchc, *Cajanus cajan* (L.) Mjllspaugh, *Lolium perenne* L., *Pennisetum clandestinum* Hochst. ex. Chiov., *Pennisetum sinese* Roxb., *Setaria sphacelata* (Schum.) Stapf. ex. Massey cv. Narok, *Setaria viridis* (L.) Beauv. and *Trifolium repens* L.

In a greenhouse study using plants grown in pots, crofton weed was shown to be less interspecifically competitive than *S. viridis* and *S. sphacelata*, when comparing their shoot height and biomass at seedling stage (Jiang, 2007). The addition of nitrogen enhanced the relative competitive ability of the forage species, which indicated that improvement of fertilization could be of additional benefit to the restoration of the pasture. Field plots studies by Jiang (2007) confirmed that the growth (i.e. biomass) of *A. adenophora* was heavily suppressed by *S. sphacelata*, when both species were planted in a mixture at different densities, but no impacts were observed on the growth of *S. sphacelata*. Compared with the biomass produced in its monoculture plots, the biomass of *A. adenophora* decreased by over 70% in the mixed plots. The relative competitive ability of *S. sphacelata* was still higher than that of *A. adenophora* after 16 months in the field. This approach has been widely used and recommended in the invaded areas for pastures and animal farming.

# Discussion

Field management of *A. adenophora* in China has been practised extensively using chemical and mechanical control, and hand removal. However, these methods are not sustainable, result in environmental problems, are labour-intensive and expensive, and still fail to contain the weed. Classical biological control was not effective probably due to the impact of the indigenous parasitoids on *P. utilis*. Biological control using fungal pathogens as mycoherbicides is still at the research stage and it is too early to say how effective they will be in the field. Recently many efforts have been made on ecological restoration of invaded pastures by native plants and forage grasses. However, ecological restoration itself is also not a sole solution to resolve the weed problem since it would not be feasible to restore all of the vast areas already invaded by the weed.

Studies on the mechanisms involved in invasion biology have also shed light on sustainable management of *A. adenophora*. It appears that *A. adenophora* colonizes human-altered environments (i.e. roads and streams) to which it is better adapted than native species, rather than invading undisturbed habitats and displacing locally adapted native species (Sax and Brown, 2000; Lu and Ma, 2006). Its invasion success is significantly negatively correlated with native plant diversity, and reduced native species cover appears to facilitate the invasion. Management to control *A. adenophora* in southwest China should also focus on habitats along roads and streams, which provide a significant source of seed and facilitate the spread of the weed into other habitats. Increasing canopy cover of native species

could help control *A. adenophora* invasion in these area (Lu and Ma, 2006).

The biological control programme against mist flower, *Ageratina riparia* (Regel) R. King and H. Robinson (Asteraceae) in Hawaii is considered one of the most successful undertaken anywhere in the world (Morin *et al.*, 1997). The most important control agent for *A. riparia* in Hawaii appears to be the fungus *Entyloma ageratinae* Barreto and Evans, followed by the gall fly *Procecidochares alani* Steyskal and then the plume moth *Oidaematophorus beneficus* Yano and Heppner. It is interesting to note that a range of parasitoids destroyed up to 50% of *P. alani* in galls in Hawaii and parasitism was the main factor limiting its effectiveness in Queensland (Morin *et al.*, 1997). Morin *et al.* (1997) suggested that the fungus and the gall fly were complementary in their activity, and that both should be introduced into New Zealand for a biological control of *A. riparia*.

With all these experiences, we believe that a sustainable management programme could be established for *A. adenophora* in China using an integrated approach, underpinned by classical biological control. New agents need to be sourced from the centre of origin of the weed (Mexico), which are complementary to the two agents already impacting (albeit mildly) on *A. adenophora*. For example, a lepidopterous and a curculionid stem borer have been identified from Mexico (Osborn, 1924) as well as *Baeodromus eupatorii* (Arthur) Arthur, a rust fungus from Central America (Buriticá and Hennen, 1980). In high value land, the use of indigenous fungal pathogens as mycoherbicides may be economically viable. Ecological restoration with native plants and forage grasses, integrated with other measures such as mechanical removal, fire and chemical control, may provide the optimum approach. Over the vast areas of natural habitats, classical biological control will need to form the foundation of the integrated approach.

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# Trans-Atlantic opportunities for collaboration on classical biological control of weeds with plant pathogens

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In North America, introduced invasive weeds are having catastrophic effects on agricultural and natural, wild ecosystems. Many of these weeds have been introduced from Eurasia, and the only economically feasible means for controlling them is through classical biological control. This situation is the same in Europe with invasive weeds introduced from North America. Development of pathogens for classical biological control involves collection of pathogens in the native habitat of the target weed, testing the pathogens in quarantine in the country of prospective release or in fields in the country(ies) of origin, and release in the non-native environment. However, discovery of new pathogens has mostly been the purview of the explorer from the country with the weed problem. A more efficient approach would be for explorers from the country of origin to discover pathogens on native species and send them to the country(ies) where the weed is a problem. Given established collaboration, this would work reciprocally with pathogens being discovered in North America and Eurasia, tested in the native range, and sent to the collaborator(s) for rapid advancement of pathogens toward release. However, there are some basic requirements for this to work. Mutual advantages, requirements, and an example are presented.

# Factors affecting success and failure of *Diorhabda* ‘*elongata*’ releases for control of *Tamarix* spp. in western North America

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The 1999 cage releases and 2001 research releases of a central Asian form of the saltcedar leaf beetle, *Diorhabda* ‘*elongata*’ (Chrysomelidae) has led to successful establishment of the agent in some locations, substantial target mortality at four release sites, and numerous ‘desired’ responses by ecosystem and community elements. However, the majority of releases have resulted in limited establishment or failure. Three ecological factors appear to account for these failures: (1) this beetle from 44°N latitude enters reproductive diapause in response to daylength too early in the season for populations to establish at latitudes lower than ca. 38°; (2) invertebrate predation can inhibit population establishment, particularly where developmental responses are not ideal; and (3) the target species in some regions is a poor quality host for the approved agents. Other ecotypes from the *D. elongata* species complex from different latitudes and bioregions may provide phenotypic traits that allow successful establishment at different latitudes and against different target plant genotypes. Five ecotypes are currently being cage-tested across three latitudinal gradients in North America to determine which develops, reproduces, and over-winters most successfully in regions where saltcedar biocontrol is desired.

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# Advances in *Striga* mycoherbicide research and development: implications and future perspective for Africa

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*Striga* spp. are important pests of cereals in semiarid, tropical Africa. An integrated approach, in which biocontrol represents an important component, appears to be an ideal management strategy for *Striga*. Our recent research focuses on the development of appropriate mycoherbicidal formulations and delivery systems to facilitate practical field application of the potential *Striga* mycoherbicides *Fusarium oxysporum* (Foxy 2 & PSM197). Hence, Pesta formulation, made by encapsulating fungal inoculum in a matrix composed of durum wheat-flour, kaolin, and sucrose, was developed. Further, a seed treatment technology for coating sorghum and maize seeds for further minimizing the inoculum amount and facilitating delivery of *Striga* mycoherbicides was provided. Both formulations showed promising efficacy in controlling *Striga*. The integration of *Striga* mycoherbicides with *Striga*-resistant sorghum and maize clearly enhanced the efficacy of both mycoherbicides in controlling *Striga* under field conditions. Further, both mycoherbicides maintained excellent viability on Pesta products and treated seeds after one year of storage, sufficient for their use under practical conditions of storage, handling, and delivery. The compatibility and suitability of Pesta and seed treatment technology for formulating and delivering *Striga* mycoherbicides can contribute to solving the primary difficulties of large-scale underemployment of *Striga* mycoherbicides in Africa. Strategies for integrated *Striga* control using mycoherbicides are proposed for African subsistence farmers.

# Multispectral satellite remote sensing of water hyacinth at small extents – a monitoring tool?

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Water hyacinth (*Eichhornia crassipes*) is widespread in South Africa, occurring in inaccessible and often small water bodies. Our objective was to determine if medium resolution (10–30 m) satellite remote sensing using existing imagery can be used to monitor the growth and health of water hyacinth populations at small extents in South Africa. Measuring the area covered by water hyacinth, as a result of classification accuracy, at decreasing resolutions was evaluated. The usefulness of satellite imagery for predicting health of plants using the Normalized Difference Vegetation Index, and the feasibility of using satellite remote sensing, were assessed. Classification accuracy is greatest on water bodies larger than 5 ha with greater than 30% cover of water hyacinth. SPOT IV imagery is the most cost-effective per image. IKONOS imagery (4 m resolution) should be used to monitor sites smaller than 5 ha; however, ground sampling smaller sites is more cost-effective. SPOT IV imagery is recommended to determine the extent of a water hyacinth mat. Seasonal or biannual monitoring is adequate to detect change based on field biomass measurements. The nature of the change in extent can be determined at sites larger than 50 ha using SPOT IV imagery, and at smaller sites using IKONOS imagery.

# Innovative tools for the transfer of invasive plant management technology

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Managing nuisance/invasive plants is an ever growing problem throughout the world today. Different methods of control (i.e. biological, chemical, mechanical, etc.) are available but information is, at times, difficult to obtain especially in one easy to access location. Innovative tools are needed that allow for rapid and efficient access to information on the identification and management of these problem plant species. Toward this goal, the US Army Engineer Research and Development Center has produced two plant management information/expert systems that allow for ready information access. These systems, PMIS and APIS, allow for information access on a variety of plant management topics including plant identification, plant biology as well as on various management techniques including an in-depth section on biocontrol. The systems come in two formats including CD and web-based versions. A new version of APIS is currently in beta testing and runs entirely on handheld personal data assistants (PDA) and smartphones using Windows Mobile technology. This mobile format allows for information access in even the most remote locations. With the availability of these systems, plant managers now have better and more efficient access to information on available technologies to manage invasive plant species.

# Physiological age-grading techniques to assess reproductive status of insect biocontrol agents of aquatic plants

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Physiological age grading is used to assess reproductive health and status of many insect species using ovarian morphology to ascertain reproductive status, reproductive history, and eggs oviposited. Three physiological age-grading systems have been developed for agents of aquatic plants including two weevil species; *Neochetina eichhorniae* and *Euhrychiopsis lecontei*, and the leaf-mining fly, *Hydrellia pakistanae*. All three systems utilize ovarian morphology to determine changes in reproductive condition. The two weevil species have similar ovarian developments/morphologies and thereby utilize almost identical systems, where changes in fat body, cuticular hardness, and follicular relics give rise to three nulliparous and three parous stages. Ovarian morphology/development differs in the fly but also uses characteristics of follicular relics to distinguish three nulliparous and four parous classes. Strategies of ovarian development differ between these species based on longevity and habitat. The weevils are long-lived and reside in relatively protected habitats and mature/deposit eggs individually throughout most of their life. The fly is short-lived, resides in open areas, and only oviposits when an entire batch or compliment of eggs are mature. Interestingly, the fly emerges as an adult with mature eggs ready for oviposition; another strategy to maximize egg production and minimize predation over a short life span.

# Use of multi-attribute utility analysis for the identification of aquatic plant restoration sites

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Presence of a diverse native plant community has been shown to enhance weed management especially in the presence of a capable herbivore. Therefore, an important consideration when designing weed biocontrol projects is the implementation of well-designed revegetation programmes. Such is the case for the management of submersed aquatic plants; for example, the greatest hydrilla declines occur in the presence of both native plants and sustained fly herbivory. While progress has been made in developing techniques for native aquatic plant culturing/planting, only limited information is available that allows non-technical personnel the ability to select suitable sites for re-vegetation efforts. To solve this problem, a decision support model was developed using multi-attribute utility analysis (MAU) where re-vegetation experts identified 11 important site characteristics ranging from shoreline gradient to sediment type. For each characteristic, utility functions were developed, which incorporate probabilities for site selection across a wide range of site characteristic values. Once the information is collected and entered, the system provides an instantaneous, prioritized listing of sites suitable for re-vegetation based on expert opinion and facts. A Web-based version has been developed allowing non-technical personnel easy and efficient access to this important aquatic plant revegetation site selection tool.

# Induced resistance in plants – friend or foe to biological control?

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Over the last 10 years, research into induced resistance (IR) in plants to pathogens and insects has developed from a theory with a little empirical evidence to a major field within plant products identified. Does IR affect biological control? To date, reducing the effects of IR on biocontrol agents has only been considered in a few cases, in which inhibitors of plant defence mechanisms have been combined with mycoherbicides. Yet IR could have a wide effect, especially with multiple sequential releases (To what extent is the interspecific competition and interference often recorded here due to IR?), or when biocontrol agents are introduced to indigenous plants that already have some damage, and thus may already be induced. Can adjuvants already used in mycoherbicide formulations stimulate or inhibit IR?

These and other questions will be addressed in this paper, which seeks to raise awareness of this topic by giving an overview of IR studies in weeds, its potential affects on biocontrol organisms, how this can be managed, and whether IR can be harnessed to the benefit of biological control.

# Turning the tide – using the sterile insect technique to mitigate an unwanted weed biocontrol agent

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The most successful programme of classical biological control of weeds has been the control of invasive prickly pear cactus (*Opuntia* spp.) by the Argentine cactus moth *Cactoblastis cactorum*. However, the moth has now become an invasive pest in the southeastern USA and its ability to dramatically control its host plant raises concerns for the safety and survival of the many ecologically, agriculturally, and culturally important *Opuntia* spp. in southwestern USA and Mexico. The sterile insect technique (SIT) has been developed for this insect as an areawide control measure. A validation/implementation study of the SIT coupled with sanitation efforts (removal of eggsticks, infested pads/larvae, and pupae) has limited the western spread of the moth. Sterile insects released in the field were highly competitive against wild moths. Competitiveness was evaluated for males by their recapture rate in pheromone-based monitoring traps and the proportion of sterile eggsticks produced as a result of sterile males mating with wild females. Continued refinement of the SIT against *C. cactorum* represents an opportunity to manage this biological control agent become pest. If implemented rapidly on new introductions, SIT can also serve as an effective risk management tool to eradicate other new invasive pests.

# Integrated weed control using a retardant dose of glyphosate: a new management tool for water hyacinth

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Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach has a major impact on aquatic ecosystems in South Africa despite biocontrol, which remains hampered by high nutrient levels and low temperatures. Often, the biocontrol agents are unable to overcome rapid weed growth, necessitating the need for intervention by herbicidal control. However, lethal doses of herbicides have harmful environmental consequences and kill the biocontrol agents by removing their habitat. The weed resurges from seed or overlooked individuals. However, glyphosate sprayed at a 0.8% concentration retards the growth and vegetative reproduction of the weed without detrimental effects on the biocontrol agents. High nutrient levels and season did not override the retardant effects of the herbicide. If spray volumes are adjusted to plant biomass, this method offers a low impact integrated weed management tool for weed-affected water systems.

# Avoiding biotic interference with weed biocontrol insects in Hawaii

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Equable climates in tropical habitats favor high year-round populations of generalist predators, parasitoids, and pathogens, which may hamper establishment and limit population growth of insects released for weed biocontrol. In Hawaii, the many past introductions for insect biocontrol as well as accidental introductions of herbivore enemies have led to awareness of problems with biotic interference in weed biocontrol. A variety of strategies are available to help predict and avoid biotic interference with future introductions. Matching checklists of Hawaiian arthropod enemies against enemies of prospective biocontrol agents known from their areas of origin provides a basis for early prioritization of candidates. For our most promising agents, we conduct pre-release quarantine tests to detect vulnerability to selected enemies. Retrospective studies also are useful: evaluating populations of lepidopteran agents released in the past helps us better understand impacts of natural enemies such as egg parasitoids. Field studies with chrysomelids provide data on potential enemies of a candidate agent in this family, which has been seldom used for biocontrol in Hawaii. New approaches to studying multitrophic interactions of herbivores will contribute other tools for predicting and avoiding barriers to successful weed biocontrol.

# Sustainable management, based on biological control and ecological restoration, of the alien invasive weed, *Ageratina adenophora* (Asteraceae), in China

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Crofton weed, *Ageratina adenophora* (Asteraceae), originally from South America, was introduced into China in the 1940s. The weed distributed rapidly and is seriously damaging grasslands and livestock production in southwestern China. To tackle the weed problem for sustainable use of pastures, an integrated strategy, based on biological control and habitat restoration, is being explored. In 1983, a gall fly *Procecidochares utilis*, originating from Mexico, was introduced from Tibet into Yunnan Province for the control of crofton weed. The current efficacy of this agent was investigated, but no significant control effect was found and heavy parasitism (70%) by native wasps was responsible. Surveys were undertaken to investigate indigenous fungal pathogens infecting the weed. Three strains of *Alternaria alternata* and a *Pestalotiopsis* sp. were isolated from leaves; a strain of the former species (YN01) was selected for further study as a prospective mycoherbicide. Surveys in Mexico to look for potential new classical biocontrol agents are planned for 2007. Field trials on ecological restoration by competition replacement of native plants and forages, under different fertility and plant density ratio conditions, were conducted. The results are discussed together with planned work on the interaction of natural enemies and this restoration approach.

# Biological control of emerging weeds in South Africa: an effective strategy to halt alien plant invasions at an early stage

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Biological control of incipient or emerging weeds (plants in the early stage of invasion) is internationally an uncommon practice due to restricted research funds being allocated to weeds that have already reached detrimental levels. Previously in South Africa, because of limited funds and, as a result, few opportunities to conduct overseas exploration work, researchers have capitalized on their survey trips by collecting potentially useful biological control agents from as many target plants as possible. Earlier exploration for agents against high-priority weeds thus allowed simultaneous collection of natural enemies of low-priority weeds in the same region. Such opportunistic programmes have been beneficial for South Africa in the management of several emerging weeds. Formal classification systems, however, have since been developed in South Africa for the prioritization of invasive alien plants. In light of this, the 'Working for Water' Programme, the main funding agency for weed biological control research in South Africa since 1996, officially recognized and awarded funds for five emerging weed programmes in 2003. This paper reviews the cases where emerging weeds were targeted for biological control in South Africa and where successes were achieved; the use of classification systems to prioritize the management of invasive plants, and the progress achieved with the emerging weed programmes currently underway in South Africa.

# Routine use of molecular tools in Australian weed biological control programmes involving pathogens

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Molecular techniques are increasingly being utilized in classical biological control programmes for weeds to characterize agents and targets and streamline the agent selection process. They are now routinely employed during the development and implementation of weed biological control programmes involving rust pathogens in Australia. For example, molecular characterization of pathogens has been used to confirm identification of a potential biocontrol candidate (*Puccinia lagenophorae* on *Senecio madagascariensis*), determine the genetic diversity of an illegally introduced pathogen before considering introduction of additional strains (*Puccinia xanthii* on *Xanthium occidentale*), and examine intraspecific genetic and virulence variation of a rust fungus to assist selection of an appropriate strain for release (*Puccinia myrsiphylli* on *Asparagus asparagoides*). Molecular diagnostic tools have been investigated as a means to confirm establishment of new pathogen strains released in areas where populations of the pathogen already exist, as strains cannot be differentiated from each other using morphological characters (*Phragmidium violaceum* on *Rubus fruticosus* agg). Molecular detection based on polymerase chain reaction amplification will also be used to confirm establishment after release of a systemic rust pathogen that has a long incubation period before visible symptoms develop on plants (*Endophyllum osteospermi* on *Chrysanthemoides monilifera* subsp. *monilifera*).

# An ecological approach to aquatic plant management

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A simple, yet often used concept of integrated pest or plant management (IPM) is one where all available management options are considered as part of a toolbox or arsenal. These 'tools/weapons' are then used singly or in combination in an effort to maximize control without impacting the use of one or more strategies. While this approach can be effective, it tends to provide only short-term control by neglecting the underlying reasons for the formation of the infestations. A more prudent and ecologically compatible approach would be the use of an ecosystem-based IPM programme that relies heavily on ecosystem management and restoration strategies and addresses causative factors that allow such formations. A key component of an ecosystem approach to managing aquatic plants is the use of host-specific biological control agents. Most of the economically important invasive/nuisance aquatic plants are introduced species that have escaped their host-specific herbivores and pathogens. In addition to their high intrinsic rates of increase, this lack of sustained feeding and resultant damage allows the formation of extensive monospecific infestations. By re-establishing a complex of host-specific herbivores and pathogens and implementing revegetation using native plants, these invasive species can be held at non-problem levels.

# A cooperative approach to biological control of *Parthenium hysterophorus* (Asteraceae) in Africa

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*Parthenium hysterophorus* (Asteraceae) is a serious invader, impacting on agriculture, biodiversity, and human and animal health in Africa, Asia, some Pacific Islands and Australia. Dense infestations are present and spreading in large parts of eastern and southern Africa and islands off the mainland. Despite decades of significant control efforts against parthenium in Australia, and to a lesser degree in India, no biological control programmes were initiated on this species in Africa until 2003, when natural enemies were introduced into South African quarantine in a programme targeting emerging (or incipient) weeds. Three damaging insect species and a pathogen that target flowers, stems, and leaves were imported. In 2005, a cooperative project was initiated for the management of parthenium in eastern and southern Africa, under the auspices of USAID IPM CRSP, with South Africa and Ethiopia the foci for biological control programmes within each region. The project encompasses host range studies, assessments of agent impact, and transfer of technology regarding appropriate facilities and training for weed biocontrol research. Additionally, studies of parthenium distribution, socioeconomic and biodiversity impacts, and methods for improved pasture management are being undertaken. The value of international cooperative research programmes is discussed.

# Biological control of *Asparagus asparagoides* may favour other exotic species

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Environmental weeds can have an impact on native biodiversity and nutrient cycling. The aim of biological control of environmental weeds is to reduce these impacts and restore ecosystem health. A biological control programme needs to not only evaluate agent establishment and subsequent decrease in the target weed but also determine if the impacts of the weed have been reduced. This is a difficult task because few studies on weed impacts are undertaken before biological control commences. This is not the case for the weed *Asparagus asparagoides* (bridal creeper), which is targeted for biocontrol within Australia. *A. asparagoides* invasion has resulted in a decrease in number and cover of native plants. Areas of low species richness can be susceptible to weed invasions and therefore any reduction in *A. asparagoides* may result in the expansion of other weeds. Invaded areas also contained elevated soil nutrients, which in Australia favours exotic plants over natives. It has been suggested that restoration efforts should be dealt with post-control and is therefore a separate issue to biocontrol. However, our study clearly demonstrates that biocontrol must be coupled with other restoration techniques.

# The past, present, and future of biologically based weed management on rangeland watersheds in the western United States

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Saltcedars (*Tamarix* spp.) are exotic, invasive perennials introduced into the western USA from Eurasia, and are among the most damaging weeds in western riparian habitats. Here we provide an update of *Tamarix* spp. control by *Diorhabda elongata* in the Intermountain West, and describe future research plans for the USDA-ARS Exotic and Invasive Weeds Research Unit in Reno, NV, USA. Our goal is to develop ecologically sustainable means of suppressing saltcedars and other exotic, invasive weeds of the region. We have adopted a 'weed management pipeline' approach that integrates classical biological control with ecological studies, aimed at maximizing the beneficial effects of biological control agents while minimizing their potential detrimental effects on the soil and native flora and fauna. This work includes use of hyperspectral imaging and other tools to characterize the spatio-temporal dispersal and impact of biological control agents on a region-wide, long-term scale. Other studies will address ecological interactions between biological control agents and their natural enemies, and the effect of plant–insect interactions on plant ecophysiology and hydrology. Successful control of a target weed usually requires decades of research; the proposed studies will be an important step toward ecologically rational management of some of the most important weeds in the western USA.

# An adaptive management model for the biological control of water hyacinth

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As many sites affected by water hyacinth are inaccessible or remote, managers require a weed management guide that does not necessitate detailed and expensive monitoring. Here we focus on two site-specific questions: What will the water hyacinth population look like if no control measures are used? What type of control will classical biological control using *Neochetina* spp. provide? We developed management recommendations for a given set of abiotic conditions based on an extensive review of experimental and field observations. In tropical situations, given time, water hyacinth should be well controlled by water hyacinth weevils, but in subtropical and temperate regions, there are many conditions under which water hyacinth still prospers, but weevils provide little control. The recommendations produced were found to agree with the preliminary results of a long-term multi-site monitoring project in South Africa. In addition to providing a readily accessible guide for managers, the model presented here also creates a focus for the development of new control methods and an assessment of integrated control options.

# Monitoring garlic mustard populations in anticipation of future biocontrol release

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Garlic mustard (*Alliaria petiolata*) is native to Europe, but has become invasive in forested regions throughout the United States. Garlic mustard is a concern because of its ability to invade high-quality forests, form dense populations, and decrease abundance of native species. The evaluation of potential biocontrol agents may result in the availability of *Ceutorhynchus* weevils for biocontrol. Accurate and well-designed monitoring is essential to provide data as to the success of the biocontrol agents and the status of the ecosystem. Monitoring data can be used to determine if the target species has been reduced and if the native species are returning. Garlic mustard is a biennial and its populations can vary from year to year. Early monitoring is necessary to accurately characterize the population before biocontrol release. Two years of garlic mustard monitoring data from 12 sites has provided information about garlic mustard population dynamics, a characterization of the plant communities associated with garlic mustard, and a documentation of the low levels of herbivory currently found on garlic mustard in Minnesota (USA). Pre-release monitoring is an important component of biocontrol release.

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# Workshop Reports

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# Feasibility of biological control of common ragweed (*Ambrosia artemisiifolia*) a noxious and highly allergenic weed in Europe

**Conveyors: D. Coutinot,[1] U. Starfinger,[2] R. McFadyen,[3] M.G. Volkovitsh,[4] L. Kiss,[5] M. Cristofaro[6] and P. Ehret[7]**

## Introduction

Established in Europe for more than a century, the common ragweed, *Ambrosia artemisiifolia* L., is the most polluting invasive weed causing allergies to European populations in various countries and is causing enormous health costs. At present, there is no effort from European decision makers to implement biological control at the European level against common ragweed.

### Presentation of results of the international meeting of experts, Vienna (AGES), 27 September 2006 (Uwe Starfinger)

On the invitation of BBA, Braunschweig, and AGES, Vienna, experts from the fields of agronomy, botany, ecology, plant protection and road maintenance from seven European countries gathered for a one-day workshop to discuss the problems caused by *Ambrosia artemisiifolia* and the availability and effectiveness of control measures. Results and individual contributions are published at the BBA website (www.bba.bund.de/ambrosia). In particular, the experts

- reported impacts of *A. artemisiifolia* in several European countries on human health, plant health and nature conservation,
- expressed their concern about an ongoing spread of the species in Europe,
- urged authorities in countries concerned to prevent further import and spread or to control existing populations,
- gave a set of recommendations for all private or public bodies concerned.

The experts considered biological control as an important tool in the strategy of managing *A. artemisiifolia*:

- *A. artemisiifolia* is a suitable target for biological control in natural, ruderal and settlement areas, as well as along traffic ways.
- From a risk assessment point of view, the sub-tribe Ambrosiineae does not include crop species in Europe.
- The risk to *A. maritima*, the only native species of the sub-tribe in Europe, has to be assessed.
- Success of biological control of a closely related species (*Parthenium hysterophorus*) and *A. artemisiifolia* in Australia is a good argument to establish biological control in Europe.
- Additional suitable biological control agents have been identified in the native area of *A. artemisiifolia* and have to be investigated further.

A follow-up meeting is planned for 2008 at the next NEOBIOTA conference in Prague.

### Success of biological control against common ragweed in Australia (Rachel McFadyen)

*Artemisia artemisiifolia* is classified as a declared 'class 2' weed in two eastern states of Australia: 'can potentially cause substantial economic and environmental damage and land managers must take reasonable steps to keep their land free of these weeds.' Biological controls

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have been implemented as a management option for ragweed in Australia. Several insect species have been introduced for biological control of common ragweed, two of which are established and giving good control. The leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) was introduced from Mexico in 1980 as a biocontrol agent for the weed *Parthenium hysterophorus* L. (Asteraceae). Defoliation has immediate negative effects on plant performance and as a consequence affects the growth, reproduction and fitness of the plants. In some locations and years, *Z. bicolorata* caused 85–100% defoliation, resulting in significant reductions in plant density, growth and flower production. East coast areas receiving greater and more frequent rainfall had higher weed density and consequently higher beetle density. The stem-galling moth *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae) was introduced from Mexico in 1982 for biological control of *P. hysterophorus* and became widely established within 2 years of introduction. *E. strenuana* now occurs throughout the range of its host plants parthenium, Noogoora burr (*Xanthium occidentale* Bertol.) and annual ragweed (*A. artemisiifolia* L.) in Australia. Galls can kill seedling plants and severely reduce growth of larger plants, and as a result annual ragweed is no longer a serious weed in eastern Australia.

## Biological control of common ragweed, *Ambrosia artemisiifolia* L. in Russia (Mark Volkovitsh)

The ragweed leaf beetle, *Zygogramma suturalis* F. was introduced to Russia from the USA and Canada in 1978 to control the common ragweed, *Ambrosia artemisiifolia*. The initial phase of this introduction was a population explosion with more than a 30-fold yearly increase in number and population density (up to 5000 adults/m$^2$ in aggregations).

In 2005–2006, we conducted selective quantitative sampling over the whole area infested by *A. artemisiifolia* in southern Russia. The average population density of the ragweed leaf beetle was very low: 0.001 adults/m$^2$ in crop rotations and 0.1 adults/m$^2$ in more stable habitats. In an overwhelming majority of inspected populations, the impact on the targeted weed was negligible. However, having regard to the spectacular success achieved in the permanent experimental plot in 1983–1985, it is still possible that stable protected field nurseries could be a promising method of *Z. suturalis* propagation for biological control of ragweed in surrounding areas.

## List of the known insect biological control agents (Dominique Coutinot)

Many potential insect biological control agents exert a pressure on *Ambrosia* in its native environment in North America. Certain of these have already been introduced and established within the framework of a classical biological control against *Ambrosia* in various countries but never in Europe. The list of all released biological control agents was presented during the workshop; the list below is those who have been reported as established:

*Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae)

Introduced from Mexico in 1982 in Australia where it is widely established.

Introduced from Mexico in 1991 in China: under evaluation.

*Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae)

Introduced from Mexico in 1980 in Australia where it is well established.

*Zygogramma disrupta* Rogers (Coleoptera: Chrysomelidae)

Introduced from the US in 1990 in Russia; the species is under evaluation.

*Zygogramma suturalis* (Fabricius) (Coleoptera: Chrysomelidae)

Introduced from Canada & US in 1978 in the Republic of Georgia and released in Abhazia (West Georgia) and Lagodekhi region (East Georgia): the establishment needs to be confirmed and evaluated.

Introduced from Canada & US in 1978 in Ukraine: establishment is not confirmed. Introduced from Canada & US in 1978 in Russia, where the species is established. Introduced from US in 1985 in the ex Yugoslavia, where establishment needs to be evaluated.

Introduced from Canada in 1988 in China, where it was recovered in some provinces. Introduced from US in 1980 in Australia, but not established.

The use of insect biological control agents within the framework of a classical biological control program against *Ambrosia* does not seem to be under consideration to date by decision-makers in Europe.

## List of potential fungal biological control agents for *Ambrosia artemisiifolia* & Species under consideration in Hungary (Levente Kiss)

There are several fungal pathogens of common ragweed (*A. artemisiifolia*) that have already been considered as potential biological control agents of this noxious weed. *Protomyces gravidus*, causing an endemic stem gall disease on giant ragweed (*A. trifida*) in the USA, was evaluated as a potential mycoherbicide against both giant and common ragweed. A *Phoma* sp.

isolated from *A. artemisiifolia* in Canada performed well in inundative experiments alone and especially in combination with a leaf-eating beetle, *Ophraella communa*. *Puccinia xanthii*, a microcyclic autoecious rust reported to infect common ragweed in some parts of the USA, was proposed as a classical biological control agent outside North America. To our knowledge, none of these, or any other fungal pathogens, are currently investigated as potential biological control agents of *A. artemisiifolia* except a strain of *Sclerotinia sclerotiorum*, which is under consideration as a mycoherbicide in Hungary.

## Biocontrol of *Ambrosia artemisiifolia* in Hungary: ideas, options and problems

Currently, Hungary is the country where the air is most polluted with ragweed pollen in Europe. Agricultural and other control measures have not helped to reduce the amount of this airborne allergen so far. Classical biocontrol of ragweed could be a feasible way to achieve this goal because most of the natural enemies of *A. artemisiifolia*, known to occur in its native areas in North America, are missing from Europe. *Puccinia xanthii*, a microcyclic autoecious rust reported to infect common ragweed in some parts of the USA, as well as two leaf-eating beetles, *Zygogramma suturalis* and *Ophraella communa*, have already been proposed as classical biological control agents of ragweed in Hungary. In addition, a mycoherbicide product based on a strain of *Sclerotinia sclerotiorum* is under development in Hungary.

## Species under consideration in Italy (Massimo Christofaro)

Ragweed, *Ambrosia artemisifolia*, an alien weed that originated from America, is now infesting the Po Valley regions in Italy. Due to the regional distribution of the plant, Italy lacks national legislation for the prevention and control of *A. artemisiifolia*. In northern Italy, regional governments as well as single municipalities have issued local laws and guidelines that establish:

- mandatory mowing in late June, late July and mid August
- general guidelines for early warning in urban areas,
- improvement of the pollen monitoring station system.

In the Lombardy region alone, the direct costs of *A. artemisiifolia* allergies on the public health system exceed 1 million euros (2003).

Mainly because of its alien origin and the territory occupied, biological control can be considered a feasible approach. The following specific steps would be required:

- evaluation of of *A. artemisiifolia* distribution in Italy,
- genetic characterization of weed and insect populations in Italy and North America,
- host specificity studies of the potential selected candidate arthropods,
- selection of one or more (specific) plant-pathogens (fungi),
- insect–pathogen interaction studies,
- evaluation of the impact of potential agents on the weed in a confined field environment,
- evaluation of potential synergisms with herbicides,
- development of an information and educational network.

In conclusion, real eradication (or at least suppression) of the target weed can be achieved only if Italy becomes involved together with other European Countries in an integrated network, using biological control in combination with other different agronomic and mechanical strategies.

## Phytosanitary status of *Ambrosia artemisiifolia*— a few examples (Pierre Ehret)

Experts participating on the workshop to discuss the problems caused by *Ambrosia artemisiifolia* in Vienna in 2006 recommended that 'countries should explore legal possibilities to regulate Ambrosia'.

It is clear that, because of its extremely large geographic distribution, this weed does not fit with quarantine regulations in most parts of the world. Different types of 'official control' and different levels of decision (national, regional or local) do exist nevertheless. Some are in the phytosanitary field; more are related to health or more global environment fields.

Local or regional approach is well adapted to an already widespread weed, and biological control seems to warrant further exploration as part of the portfolio of control methods.

# Discussion

Biological control could be one of the tools to control common ragweed in Europe. Taking advantage of the examples from Australia and Russia with insect biological control agents, further investigation and research on rust and other weed pathogens needs to be developed at a European level. Although the total eradication of this plant is not feasible, a European program for the biological control of common ragweed would be highly desirable.

This workshop had as objective to reply to the conclusions of the experts at the workshop organized in Vienna by OLD (AT) and BBA (OF) at the NEOBIOTA conference. The presentations and conclusions provide a basis for forthcoming discussions in future scientific or political conferences on the management of common ragweed including the feasibility of a biological control program against *Ambrosia* in Europe.

# Rearing Insects

**Conveyors: R. De Clerck-Floate[1] and H.L. Hinz[2]**

## Report

This informal workshop explored some of the challenges experienced in rearing insects for classical weed biological control. In a recent global survey of weed biological control, researchers revealed that problems in rearing were providing significant obstacles during biological control programs, particularly during the early stages of development (e.g. host-specificity testing). Through a mutual sharing of some of the difficulties encountered, and also of some of the novel rearing solutions that have been developed, it is hoped that ongoing and future programs can be improved, and perhaps difficult agents with biological control potential may get a second chance.

During the workshop, it was suggested that it would be useful to have a forum where researchers could exchange information on insect-rearing techniques, problems and solutions. Alec McClay volunteered to set up a forum page at http://mcclay-ecoscience.com/phpBB2/viewforum.php?f=2.

Anyone can read the forum, but to post or reply to messages you will have to register. To do this, click on the 'Register' link at the top right of the page and provide the information requested. To reduce spam, Alec has set the site up so that all registrations must be manually approved by the moderator (Alec). Please provide information in the 'location', 'occupation' and 'interests' section so that he can identify you as someone involved with weed biological control or with an associated field of work. He would prefer you to use your real name as your username or signature and will try to approve registrations soon after he receives them.

Please let Alec (biocontrol@mcclay-ecoscience.com) know if you have any problems or any suggestions to make the forum more useful, and feel free to forward this information to other weed biological control researchers.

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# Correction to a paper published in the *Proceedings of the XI International Symposium on Biological Control of Weeds, Canberra Australia*, page 121: Population structure, ploidy levels and allelopathy of *Centaurea maculosa* (spotted knapweed) and *C. diffusa* (diffuse knapweed) in North America and Eurasia

**Ruth A. Hufbauer,[1] Robin A. Marrs,[1] Aaron K. Jackson,[2] René Sforza,[3] Jorge M. Vivanco[4] and Shanna E. Carney[5]**

## Correction

In Hufbauer *et al.* (2004), the root squash technique used to gather the data for Figure 2 did not provide adequate resolution. Other collections and approaches suggest that *C. diffusa* (diffuse knapweed) within North America are diploid (Ochsmann, 2000; Blair, personal communication) and that *C. maculosa* (spotted knapweed) within North America are tetraploid (e.g. *C. stoebe micranthos*) (Müller, 1989; Ochsmann, 2000; Müller-Schärer, personal communication). Additionally, the approach used to assay catechin levels produced by individual plants for Figure 3, which followed Bais *et al.* (2002), is not possible biochemically (Blair *et al.*, 2005). Research by Blair *et al.* (2005) shows that spotted knapweed produces an average of 0.29 µg/ml (+/−)-catechin (Blair *et al.* 2005) or 0.15µg/ml (−)-catechin when grown following the protocol used here. Figure 3 also contains two typos: the units for the *y*-axis should be microgram (µg) rather than gram (g) and the extraction was from 500 µl rather than 500 ml. No data are currently available on catechin production by diffuse knapweed or hybrids between diffuse and spotted knapweed.

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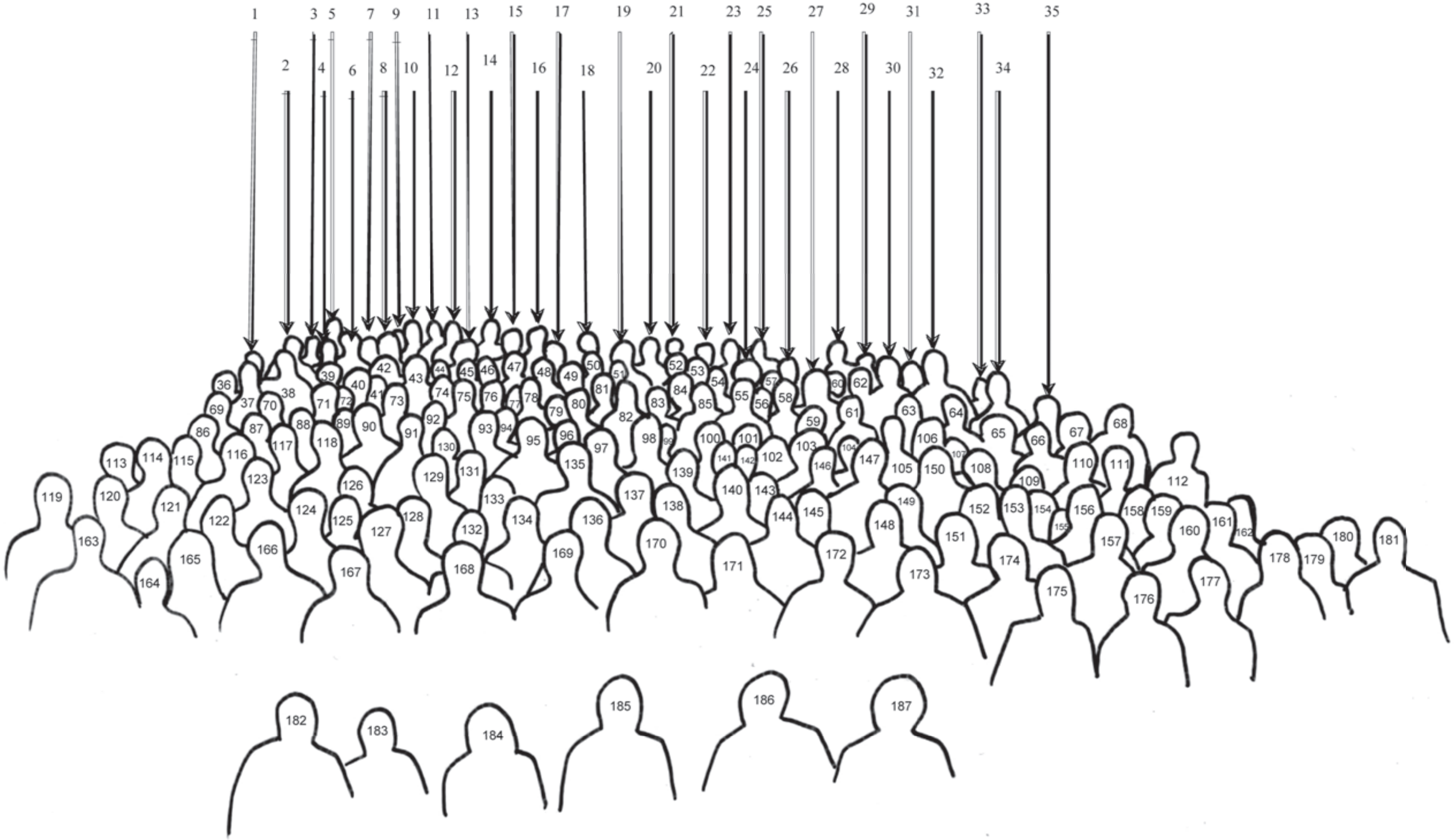


Key to symposium photograph

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| 181 | Darren Kriticos |
| 182 | Abuelgasim Elzein |
| 183 | Marie-Claude Bon |
| 184 | Janine Vitou |
| 185 | Brian Rector |
| 186 | Mic Julien |
| 187 | René Sforza |